

Second International Symposium on  
**Biomolecular Archaeology**

Stockholm, Sweden, 7–9 September 2006



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## Biogeochemical approaches offer new perspectives on old problems

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Almost thirty years ago, the first application of stable carbon isotope analysis to archaeological human material challenged conventional ideas about the adoption of maize-based diets in Woodland North America (van der Merwe & Vogel 1978). The results prompted archaeologists to re-examine the evidence for the adoption of agriculture among these groups, resulting in findings of earlier domestication of other, previously overlooked crops. This seminal study remains a notable example of the opportunities offered by approaches based on the isotopic composition of bones and teeth, since it provides reasonably direct evidence for actual consumption by individuals. But how precisely can we interpret this data, which concern the precise nature of the relationship between nutrient and isotopic composition of food, and bone/tooth collagen and mineral remains of the consumers? How well do these isotopic signals survive in the archaeological record? And to what extent can we extract, and rely on, evidence from individuals, since this is, after all, one of the distinct potential advantages of isotope tools? To a large degree we are still grappling with these challenges. Here I review progress in understanding isotope systematics in foodwebs and tissues to promote better interpretation of modern and early human diets, as well as issues related to population-scale (averages) or individual-scale (life history) dietary interpretations. The material relies partly on more recent archaeological material as well as some of the results from an ongoing programme to understand the dietary palaeoecology of Pleistocene hominins.

# Detecting milk in the palaeodiet with calcium isotopes

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We present the first results on archaeological human bone calcium isotopic compositions ( $\delta^{44}\text{Ca}/^{42}\text{Ca}$ ) which show a small but highly significant distinction between humans known to have had milk available (i.e. European Neolithic and Iron Age) and those presumed not to have consumed milk (i.e. European pre-Neolithic). The method relies on the calcium contained in milk being isotopically discriminated during metabolism from other dietary sources of calcium. Also, that levels of Ca in milk are so high that consumption of milk or its products is liable to provide most of the calcium for consumers where dairying is important. The paper will outline current understanding on Ca isotopy in food chains and metabolism, and describe the data in support of our interpretation, including addressing issues such as biological variability and bone diagenesis.

# Pottery use and the transition to agriculture in Southern Scandinavia: New evidence from organic residue analysis.

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The transition to agriculture in Northern Europe has recently described as an “abrupt” and even “traumatic” event and one where newly produced foods rapidly replaced wild ones. Interestingly however in many parts of Northern Europe, especially Southern Scandinavia and along the Baltic coast of Northern Germany, there appears to be some continuity in settlement patterns, food procurement strategies and material culture across the transition. Whilst the arrival of newly domesticated foods, such as cattle and wheat, are clearly documented from c. 4,000 cal BC, the degree of continuity in food acquisition strategies, consumption practices and dietary compositions has been avidly disputed. In search of a new perspective on this topic, here we consider how pots were used by both pre-agricultural groups (Ertebølle culture) and the first farmers (Funnel Beaker culture or TRB) in this region. Ceramic vessels were obtained from a number of classic Ertebølle, TRB and transitional sites and were subjected to a range of analytical techniques to determine the composition of absorbed and surface residues. Our approach focused on the analysis of lipid residues using GC, GCMS, GC-c-IRMS. In many cases bulk carbon and nitrogen isotope values were also obtained for comparison with the more specific lipid analyses. Many of the samples came from submerged sites which has led to excellent preservation of the lipid residues. Our results provide new insights into the types of foods which were used during the late Mesolithic Ertebølle period and describe some important changes in pottery use associated with the arrival of pastoralism.

# Collagen turnover in the adult femoral mid-shaft modelled using environmental radiocarbon tracer measurements

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We have measured the <sup>14</sup>C content of femoral mid-shaft collagen to determine the dynamics of adult collagen turnover, using the sudden doubling and subsequent slow relaxation of global atmospheric <sup>14</sup>C content due to nuclear bomb testing in the 1960s and 1970s as a tracer. <sup>14</sup>C measurements were made on femoral mid-shaft collagen from 67 individuals of both sexes who died in Australia in 1990-1993, spanning a range of ages at death from 40 to 97, and compared to values predicted by an age-dependent turnover model. We found that the dataset could constrain models of collagen turnover, with the following outcomes: (a) Collagen turnover rate of females decreases, on average, from 4% to 3% from 20 to 80 years. Male collagen turnover rates average 3% to 1.5% over the same period. (b) Much of the variation in residual bomb <sup>14</sup>C in a person's bone can be attributed to individual variation in turnover rate, but of no more than about 30% of the average values. (c) Before age 20, male collagen turnover rate appears to be significantly greater than female rate, by up to a factor of 2. For both sexes the collagen turnover rate during adolescent growth is much higher (10 – 20% at age 10-15). The implications for radiocarbon dating and palaeodietary interpretations of human isotopic values will be discussed.

# Flashes in the frying-pan? On the culture of food and the interpretation of ancient subsistence data.

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The study of ancient food habits often includes scientific or technical analyses of some sort, e.g. osteological, plant macrofossil, bone chemical or organic chemical analyses. Quite a lot of effort has been put into understanding the formation processes and post-depositional changes such as decomposition and taphonomy. The cultural aspects of the formation of these materials are often not considered. This paper is a presentation of a food culture model and some of its consequences on the interpretation of various subsistence data from ancient material remains.

# Pots and perfumes: Luxury consumption in the Late Bronze Age eastern Mediterranean?

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Transport or storage vessels of Red Lustrous Wheelmade ware (RLWm ware) are found in small quantities in tombs, temples and occasionally domestic contexts across a wide area of the eastern Mediterranean during the Late Bronze Age (LBA). NAA and petrography have shown that the fine, red fabric of the vessels is extremely consistent over the whole 300 years of its manufacture, leading archaeologists to assume a single source. Wherever it has been excavated the pottery has been classified as foreign or exotic. No kiln sites have ever been found and the origin of RLWm ware has been a subject of debate for over 100 years.

Although very well made, RLWm ware is plain compared to other contemporary wares. This, together with its 'value-added' use in tombs has led to the conclusion that it was traded for its contents. One of the aims of this project is to examine the organic residues from RLWm ware sherds from a range of sites across the eastern Mediterranean to try and determine what commodities were transported and stored in these vessels. GC-MS analysis has so far identified two possible sealants (beeswax and bitumen) and fats or oils which probably represent the contents of the vessels – possibly the perfumed oils often suggested by archaeologists. Differences in the contents of the vessels found at different sites may provide information on the consumption of commodities by different cultures. Combined with the changes in distribution of the ware over time, these consumption patterns may also provide insights into the trade and political relationships of the LBA eastern Mediterranean and help to locate the source of RLWm ware. Variation of contents with the shape of vessel has also been suggested, with different shapes being unique packaging for a particular commodity, and this theory is also being investigated.

# Nitrogen ( $\delta^{15}\text{N}$ ) isotope fractionation: Are humans special?

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The application of nitrogen ( $\delta^{15}\text{N}$ ) isotope fractionation to trophic level interpretations and relative meat/seafood in the human diet is based on several untested assumptions. Among these assumptions are that relatively uniform and depleted values of  $\delta^{15}\text{N}$  are found in plants; that the stepwise fractionation of  $\delta^{15}\text{N}$  up a trophic level will be approximately 3‰; that “protein routes to protein”; that variability in  $\delta^{15}\text{N}$  of bone collagen from a population with roughly the same diet is known; and that metabolism has little effect on nitrogen isotope systematics. This presentation will explore each of these assumptions and discuss recent data that addresses these issues with an emphasis on the interpreting  $\delta^{15}\text{N}$  in human bone collagen.





## Anthroposols and the past: A review of current research and future opportunities

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Soil has for a long time been something for archaeologists to remove in order to reveal the secrets of the past encompassing house plans and artefacts, but in modern studies of archaeological science, with biomolecular archaeology in particular, it may be an important source material, whether it is to reveal the function of single enigmatic features, or to trace human activities in the landscape, such as settlement organization or agriculture, or to research into the environment. In addition to these traditional questions of archaeology to be answered through the medium of heavily degraded organic *material culture* in soils and sediments, the analysis of soil has since many years played a role in archaeological prospection. Though most effort was put into phosphate and trace elements analysis, the study of molecular residues of an organic origin in soil might be seen as a new challenging approach in the geochemical prospection of archaeological sites. With regard to the further development of these fields of research as well as to the modern concept of *in situ* preservation of archaeological sites, a thorough understanding of the processes of deterioration of organic materials under various soil conditions has become highly important. Gaining insight into the biogeochemical decay sequences of organic remains is badly needed, whereas the study of archaeological biomarkers, having properties that can be directly related to known biological precursors, has to be intensified. This includes the exploration of new yet unknown compounds relevant to archaeology, their research potential, and their apparent stability within specific soil types.

# Death comes to town: Tuberculosis, urbanisation and natural selection

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The interaction between infectious diseases and past human societies has been intensively studied, and yet key questions remain unanswered. Here, we discuss some of those questions, and focus on one in particular: the timing and cause of the emergence of tuberculosis (TB) in humans. TB has been, and still is, a major cause of death worldwide, and is clearly significant to our understanding of past human lifeways. Yet biomolecular approaches to the study of TB have so far proved difficult to implement at the global scale required. In order to address this problem, we have sidestepped the use of ancient DNA, and instead employed a novel approach examining DNA from modern human populations. We show that TB is associated with the growth of urbanisation, and that natural selection for TB resistance has clearly acted most strongly on populations with a longer history of urban settlement. The results support the notion that TB has become an important disease chiefly since the origins of high-density settlement, and provide a previously unrecognised example of biological adaptation to cultural change.

# Detection and characterisation of organic inputs into anthropogenic sediments from a Bronze Age Syrian royal tomb

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A total of 50 sediment samples from the floor of a Bronze Age royal tomb excavated at the archaeological site of Qatna, Syria have been obtained. The tomb itself is rock cut into a limestone cliff and has remained sealed since the destruction of the overlying royal palace by the Mittani in the mid 14<sup>th</sup> century BC. No organic material would have been present prior to its use as a tomb and therefore the sediments may be considered to be purely anthropogenic in nature, arising from the degradation of materials (structures and commodities) associated with the tomb's use. Major inputs are anticipated to be organic remains of decomposed tissues, embalming agents and treatments, food offerings, plant materials, wood and resins. A unique opportunity is therefore available to study the sediments as a sink for chemical indicators of ancient (ritualistic) human activity. This paper focuses on the results obtained from biomolecular techniques such as Pyrolysis-GC/MS and lipid extraction followed by characterisation by Gas Chromatography (GC) and GC/Mass Spectrometry (GC/MS) currently being employed in order to investigate the organic inputs to the sediment at a molecular level by screening for a variety of well characterised biomarker compounds. Such compounds include adipocere derived fatty acids and sterols indicative of the presence of human remains, phenolic compounds derived from plant remains, biomarkers for beeswax and other insect waxes along with more exotic commodities such as resins, perfumes and ointments. The most significant result to date has been the detection of 6,6'-dibromoindigo, the major coloured component of the important ancient dye Tyrian purple, in over 40% of the samples. The distribution of this compound across the tomb floor, along with its association with other biomolecules and archaeological remains will be discussed.

# An assessment of the value of soil proteomics for archaeological prospection

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Anthropogenic activities are known to leave chemical traces in the soil, primarily in the form of organic molecules released from occupation waste. Recent studies indicate that lipids, fatty acids and waxes can survive in soils for long periods of time and show the potential of these residues as archaeological biomarkers. Although some of these biomarkers can be traced back to their origin, the information yield is often limited, because many organic molecules in the soil have multiple sources, become mineralized or are heavily degraded. Proteinaceous biomarkers on the other hand hold specific information about anthropogenic action on the soil, but are generally believed to be very susceptible to degradation especially on archaeological time scales. However, research on more recent soils points out that peptides and proteins can be successfully protected from degradation by means of adsorption onto soil minerals, protein cross linking and encapsulation by refractory organic matter or various combinations of these processes. This study aims at the recovery and analysis of proteinaceous biomarkers from archaeological soils in order to assess the value of these residues for archaeological prospection. Soil samples from different archaeological contexts were subjected to time-resolved sequential extraction experiments and HPLC analysis to obtain an understanding of protein retention in our soils. Subsequently, we tested a proteomic approach to identify soil proteins. This included protein extraction, protein separation and MALDI-MS/MS analysis of (digested) proteins. Because large amounts of complex data are generated by this approach we also focussed on automated MALDI-MS peak recognition by means of algorithmic data processing. The work presented here shows preliminary results which eventually will give insight in the potential of soil proteomics as an advanced method for archaeological prospection and site interpretation.

## Diffusing culture, migrating genes: Milking the origins of Europeans

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Recent debate concerning the origins of Europeans has centred on the relative contributions of incoming Near Eastern farmers and indigenous Palaeolithic hunter-gatherers. Although genetic data can contribute to this debate and various data types and analytical approaches have been used, satisfactory conclusions remain elusive. Lactase persistence is common in people of European descent but, with the exception of some Middle Eastern and African pastoralist groups, is rare or absent in other global populations. Examination of haplotype conservation around a polymorphism that is either very strongly associated with or causative of lactase persistence in Europeans (-13.9 C/T) indicates that the derived allele is recent in origin and has been subjected to very strong positive selection. It is unlikely that lactase persistence would provide any advantage in the absence of a supply of fresh milk and this has led to a gene-culture co-evolutionary model whereby lactase persistence is only favoured in cultures practicing dairying, and dairying is more favoured in lactase persistent populations. Thus, the first lactase persistent dairying populations are likely to have undergone demographic expansion. We have developed a flexible demic computer simulation model to explore the spread of lactase persistence, dairying, other subsistence practices and unlinked genetic markers in geographic space. We find that under plausible conditions of limited gene flow between dairying and non-dairying cultures, a disproportionately high component of European ancestry will trace back to Europe's earliest lactase persistent pastoralists. Furthermore, we find that lactase persistence could have originated across a wide range of different locations in Europe and still achieve the distribution we see today.

# On the impossibility of proving the authenticity of a human aDNA sequence

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There are two ways of approaching the subject of contamination: a technical and a "cognitive" approach. While the first addresses the conditions of recovery (excavation), sampling, decontamination and laboratory processing, the latter focuses on the interpretation of data, their content and sometimes their phylogenetic meaning.

In most cases a combination of both technical and cognitive aspects is used to draw conclusions about whether a human aDNA sequence is authentic or not. In this presentation, we try to reconsider various conclusions in light of mitochondrial DNA sequence data obtained in our labs.

**Technical aspects:** we have developed a procedure that enables the efficient decontamination of superficially contaminated bones. But we have so far been unable to find a solution for washed archaeological material that is contaminated through and through. But even if a bone is contamination free, there remains a certain degree of background contamination from the lab environment. We present anti-contamination strategies developed in the Mainz lab during the last five years. However, we have to admit that while we can reduce the contamination rate of nuclear DNA down to zero, there remains a certain degree of mitochondrial human DNA contamination even under the most stringently observed lab conditions for ancient DNA.

**Interpretational content aspects:** reproducibility remains the ultimate criterion. The dogma says that authentic DNA is reproducible in most PCRs and all amplicons while contaminations are sporadic. This seems to be true in many cases, but from a theoretical point of view we can't establish its truth in each individual case. There remains a certain error rate for any individual skeleton. In some cases contamination may be designated "unlikely" and authenticity "plausible", but these terms are far from scientific proof. We will argue for a population-wide approach that accepts inevitable errors on the individual level without affecting the overall interpretation.

# Phylogeny and ancient DNA of wild and domestic pigs provides new evidence for human dispersal routes in Island Southeast Asia and the Pacific

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Human migration from mainland Asia through ISEA, Wallacea and Near and Remote Oceania was the most extensive of global dispersals during the early-mid Holocene. The routes along which humans traveled have traditionally been inferred from associated material culture, language, and human genetic signatures. However, animal translocation has also been a consequence of human dispersal events and related trade and exchange activities, and recent genetic studies of Pacific rats and pigs have demonstrated their potential to act as proxies for reconstructing patterns of human dispersal and interaction networks. In order to test existing models of Neolithic human migration into the Pacific, we extracted mtDNA from 243 wild, feral, and domestic pigs across the region, and from 23 archaeological specimens using appropriate ancient DNA methods. A further 512 pig sequences from GenBank were used in the phylogenetic analyses. As a comparison, outline analysis (a geometric morphometric technique) was carried out on third lower molars ( $M_3$ ) of museum specimens from ISEA, and on Holocene *Sus* remains from the site of Liang Bua, Flores. This represents the first application of EFT to pig dental morphology in the field of zooarcheology. Results showed two human-mediated dispersal routes through Island South East Asia (ISEA) into Oceania. A Southern Route documents the movement of domestic pigs (*Sus scrofa*), from peninsular Southeast Asia, south through the Sunda Islands into Near and Remote Oceania, where it is associated with Lapita and Polynesian migrations. A Northern Route involves the transport of East Asian pigs to western Micronesia, possibly through Taiwan and the Philippines. Ancient DNA and morphometric studies of teeth disclose an earlier human-mediated translocation of the Sulawesi warty pig (*Sus celebensis*) within Wallacea.



# The first farmers: An aDNA study on early Neolithic skeletons of Central Europe

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The process of Neolithization in Europe has played a fundamental role in European prehistory. The fields of archaeology, anthropology, human genetics and other disciplines have been marked over the past decades by a long and outstanding debate on whether cultural and ecological innovations were propagated over Europe via transfer of knowledge or whether they were introduced by colonizing farming groups. Most of the debate about the latter centers on the extent of a putative impact of “Neolithic genetic influx” on indigenous hunter-gatherers of Central Europe. By means of aDNA analysis of the mitochondrial HVS I and additional coding region markers we were able to determine mtDNA-haplogroup status of 43 Neolithic individuals associated with early farming subsistence such as the Linear Pottery culture, the Körös culture, the Alföld Bandkeramik (LBK) and neighbouring North-Eastern groups. Our results demonstrate that haplogroup frequencies differed not only among the Neolithic sample-sets but also in comparison to modern-day mtDNA data. Besides frequency shifts of single haplogroups, an unexpected high frequency of haplogroup N1a was present in the LBK samples. Moreover, haplogroups associated with the “genetic Neolithic package” were completely absent in all of our samples. Based on these direct investigations of the female Neolithic genetic *status quo*, we put forth a few hypotheses about Paleolithic vs. Neolithic origin of Central Europeans.

# The raw material of Pleistocene bone points: Cave bear bones as paleolithic resource?

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Potočka zijalka in Slovenia was the first Alpine site that produced both a rich paleontological material and clear Upper Palaeolithic artefacts. This contribution deals with raw material analysis of the rich bone point assemblage based on eye determination, mtDNA and isotopic analysis. The combined approach revealed a more variable raw material provenance than previously assumed. Bone is still the dominant raw material, followed by antler and scarce evidences of ivory. DNA and isotopic analysis suggest cave bear bone as most likely bone source for five of the six analysed specimens. The determination is enforced by a correspondence of direct radiometric dates on cave bear bones and bone points. Derived from faunal and stratigraphical evidences, the use of subfossil bone material seems most likely. The possibilities and limitations of these approaches for raw material analysis are discussed.

# Aurochs or domesticated cattle? Ancient DNA analyses of early animal husbandry in Northern Germany

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For several hundred years beginning in approximately 5450 BC, the Mesolithic Ertebølle/Ellerbek culture in the Southwest Baltic region coexisted with adjacent Neolithic cultures to the south and east of the river Elbe. During this time of coexistence, acculturation processes led to the adoption of Neolithic elements by the Mesolithic population of East Holstein. Recovered artifacts evidence regular contact with farming cultures and archaeo-palynological analyses show that agriculture may even have been practiced on a small scale. This assumption is supported by archaeo-zoological analyses showing that Mesolithic bone samples from the Rosenhof LA 58 site are from fully domesticated cattle. However, skepticism of these results has often resulted from the professed diagnostic problem of overlap in size between small female aurochs and large male cattle. In addition, morphological analyses can give no unequivocal information on whether the presumed cattle are the result of independent domestication of indigenous aurochs populations, or domesticated animals introduced through direct contact with Neolithic settlers. To contribute to clarification of these questions, we analyzed ancient mt- and ncDNA of several presumed domesticated cattle from the site Rosenhof LA 58. In addition to haplotype determination on the basis of the HVR I region within the mitochondrial d-loop, we successfully amplified parts of zinc finger genes on the X- and Y-chromosomes for sexing. The results show that all bone samples stem from female individuals. However, only one of these samples revealed a lineage that occurs in domesticated cattle. In our presentation we will point out the influences these results will have on further morphological distinctions between aurochs and domesticated cattle. Furthermore we will introduce these new facts to the long-standing debate concerning early domestication of bovines in Northern Germany.

# Origins of agriculture in Southwest Asia

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Understanding where agriculture arose is necessary for understanding early human culture. For each of the crops that were domesticated we can ask whether its wild progenitor was taken into cultivation only once or if there were multiple domestication events. Distinguishing between these scenarios can help to answer questions concerning the origins of agriculture in the Fertile Crescent. The analysis of large genetic datasets have led to the conclusion that barley, einkorn wheat and the various forms of cultivated tetraploid wheat were each domesticated once, the wheats originating from a small area of southeast Turkey and barley from the Israel-Jordan area. However, previous work at UMIST on the origins of emmer wheat demonstrated that cultivated emmer is not monophyletic and was domesticated on more than one occasion and at different geographic locations in the Fertile Crescent. The demonstration that cultivated emmer has diverse origins provides evidence in favour of the hypothesis that the transition to agriculture in SW Asia was a necessary response to a changing environment rather than the result of a chance discovery. Furthermore, simulation studies have demonstrated that the assumptions of the phylogenetic analysis applied to the large genetic datasets are flawed and result in cultivated populations with separate origins appearing to be monophyletic. Here, results demonstrating that cultivated einkorn has more complex origins than previously suggested will be presented and the implications for future genetic analyses of crop evolution discussed.



## Decomposition pathways: Too difficult to unravel?

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Degradation adds a further layer of complication to the already difficult task of interpreting fragmentary and time averaged data from the archaeological record. There may be some instances in which degradation can be effectively ignored, but in the most exceptional cases of preservation, such as the ice man 'Ötzi', considerable attention has been taken to the diagenetic history— even though in such circumstances the degradation is minor compared to normal archaeological remains. In 'routine' archaeological samples, diagenesis is less commonly considered, but this is perhaps not surprising. In the case of exceptional preservation, the mere fact of persistence and the large number of diagenetically unstable biomaterials challenges the expected norms and offers scope for investigation. By contrast 'typical' samples have arguably extremely complex, multifaceted histories, in which degradation has stripped away more evidence than it has left. Furthermore, in many cases, notably C<sup>14</sup> dating and stable isotope analysis of bone collagen, isolation and purification strategies assume that ancient protein has equivalent properties to modern samples. Degradation is effectively ignored, with few, if any, detrimental consequences. Advances in both population scale and shotgun analyses of ancient samples, has incrementally been providing data on the state of degradation of samples, and – as reflected in this conference session – there is a renewed interest in this Gordian knot of degradation. New data is beginning to tease out information regarding preferred diagenetic pathways and the state of preservation of biomolecules. Are these new strands of information going to allow us to begin to unravel the larger problem? Hopefully further study will provide some general rules to enable some aspects of diagenesis to be ignored and others to be transformed into useful rules which can be applied when selecting samples, extraction material or analysing the data.

# Miscoding lesions in ancient DNA templates

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The damage of ancient DNA (aDNA) is one of the fundamental obstacles to aDNA research. As with contamination, damage is universal to the field in the sense that is likely to affect all sample types from all environments. The resultant effects of aDNA damage can be subdivided into two groups: (i) damage that prevents PCR amplification of the recovered DNA and (ii) damages that results in the generation of error containing DNA sequences (miscoding lesions). Although the problems of damage have been recognized since the field's earliest studies, the development of a clear understanding of the rates of occurrence, and mechanisms behind this damage has been hindered, principally by the lack (both volume and range) of available sequence data on which analyses can be performed, and limitations in the technology and methodology available to actively study such damage. Characterizing and understanding of the damage load and types in ancient DNA is vital for retrieval of authentic sequences from poorly preserved samples. This is especially true when analysing low-copy number nuclear DNA. Here we report first results of analyses comparing the frequency and distribution of miscoding lesions in mitochondrial and nuclear DNA from ancient bone, teeth and coprolite samples. Secondly we discuss novel insights on the biochemical basis behind aDNA damage, that have become available following recent technological developments.

## DNA in bones – where is it, and how can we get at it?

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The abundance of samples, their relative resistance to degradation, and their long-term preservation makes bones a natural source for ancient DNA (aDNA). However, although bone has featured in aDNA studies since the early days of the field, remarkably very few studies have actually been undertaken to characterize where the DNA is located within bones, how fast it degrades, how contamination free it remains, and how to recover the highest levels of authentic DNA from bones. In this talk we present new results from quantitative Real-Time PCR analyses on datasets of both experimentally degraded, and natural old bone, that starts to provide answers to some of the above questions. Firstly we demonstrate the surprising kinetics behind the early stage degradation of PCR amplifiable DNA in samples from various environmental conditions. Secondly we provide data on the (in)efficiency of several of the most commonly used DNA extraction techniques, and show how such methods can be improved in order to increase DNA yields. Thirdly we demonstrate that the above factors are related to protein survival in bone, and thus what implications this may have for the DNA. Lastly we combine this information with other recently published findings on DNA contamination of bone to present an up-to-date summary of the relationship between ancient DNA and bone.



# Beeswax residues in Chalcolithic (6000 year old) ceramic vessels from Israel

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Cornets are enigmatic vessels that were produced exclusively in the Chalcolithic period (~4700-3500 BC) in Israel and Western Jordan. There is no definitive evidence regarding their function. We used GC and GC/MS to analyse cornets from 3 sites in Israel: a shrine at En-Gedi in the Judean Desert, a cave near that shrine and the domestic site of Grar in the northern Negev. For comparison, we also studied pedestal bowls and holemouth jars from the same sites, as well as the sediments from the sites to monitor contamination possibilities. The lipid assemblages from all the cornets are similar to one another and differ from all the other different-shaped vessels and sediments. The cornet lipid assemblage is dominated by long chain *n*-alkanes ( $nC_{23}$ – $nC_{33}$ ), fatty acids ( $C_{16:0}$ ,  $C_{18:0}$  and  $C_{24:0}$ ) with traces of a long chain alcohol (*n*-triacontanol,  $C_{30}OH$ ).  $n-C_{27}$  is predominant among the odd numbered *n*-alkanes. The relative abundances of all odd numbered *n*-alkanes are constant, whereas the relative abundances of the even numbered *n*-alkanes vary. The odd numbered *n*-alkane distribution, together with the significant presence of triacontanol and the mentioned fatty acids is consistent with the known chemical composition of beeswax. No wax esters were preserved in the extract. The presence of the even numbered *n*-alkanes is puzzling. We analyzed modern beeswax from wild hives located in Israel. As beeswax ages, its color darkens due to high amounts of the remains of brood cuticles. Brood cuticles are known to be covered with odd and even alkanes ( $n-C_{23}$ - $C_{32}$ ) and thus accumulation of cuticles changes the alkane composition of aged beeswax. Our analyses of the beeswax showed that the darker the beeswax the larger the amount of even-numbered *n*-alkanes in the lipid extract, while the distribution of the odd-numbered alkanes remains unchanged. We therefore conclude that the cornets contained beeswax and might have served as candles.

# Tracking down human contamination in ancient human teeth

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DNA contamination arising from the manipulation of ancient calcified tissue samples is a poorly understood, yet fundamental problem that affects the reliability of ancient DNA studies. We have typed the mitochondrial DNA hypervariable region I (HVR1) of the only six people involved in the excavation, washing and subsequent anthropological and genetic study of 23 Neolithic remains excavated from Granollers (Barcelona, Spain), and examined for their presence among the 572 clones generated during the aDNA analyses of teeth from these samples. 17.13% of the cloned sequences could be unambiguously identified as contaminants, with those derived from the people involved in the retrieval and washing of the remains present in higher frequencies than those of the anthropologist and laboratory researchers. This finding confirms for the first time previous hypotheses that teeth samples are most susceptible to contamination at their initial excavation. More worrying, the cloned contaminant sequences exhibit substitutions that can be attributed to post-mortem DNA damage, and we demonstrate that the level of such damage increases with the age of contaminant; contaminants that are >10 years old have approximately five times more damage than those that are recent. Furthermore, we demonstrate that in this dataset, the damage rate of the old contaminant sequences is indistinguishable from that of the endogenous DNA sequences. As such, the commonly used argument that miscoding lesions observed among cloned aDNA sequences can be used to support data authenticity, is misleading in scenarios where the presence of old contaminant sequences is possible. We argue therefore that the typing of those involved in the manipulation of the ancient human specimens is critical in order to ensure that generated results are accurate.

# Destruction of archaeological bone: The role of specific bacteria in experimental degradation of 700 year-old human bone

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The reason why buried human skeletons can sometimes survive intact in forensic/archaeological contexts is poorly understood. The extent of degradation in skeletal remains clearly depends on the environmental conditions of burial, with a range of effects observed from virtually none to complete destruction. Although microbes are undoubtedly responsible for structural degradation of bone over time, almost nothing is known of the destructive effects of specific bacteria on individual bones. For many years it was supposed that fungi were primarily responsible for destructive processes (tunnelling) within bone but over the last 30 years the role of bacteria has been increasingly recognised. Bone sections from human femur approximately 700 years old were prepared and inoculated with a 10µl of a fully grown culture of *Prevotella intermedia* NCTC1370 in culture broth. Samples and controls with inoculants were incubated anaerobically at various temperatures. Thin bone sections (60 - 100µm) were prepared with a saw microtome and examined unstained using light and polarised microscopy. Controls were uninoculated or broth only bone sections. Despite some degradation in unexposed bone sections as evidenced by histological index analysis, more extensive degradation consistent with linear longitudinal and lamellar tunnelling was observed in sections examined by light and polarised microscopy at least 33 weeks after exposure to *P. intermedia*. The effect of varying incubation temperature on experimentally inoculated bone was also assessed. In a small preliminary study, the anaerobe *P. intermedia* appears to play an unexpected role in 'tunnelling' of archaeological human bone. Extensive microscopic focal destruction was noted in human bone sections experimentally inoculated with *P. intermedia* NCTC1370, and less so from sections not inoculated. Further studies have been initiated to determine whether this effect can be extended to other bacteria commonly found in humans and soil.

## Methodological studies on ancient DNA and ancient DNA methods

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When *World Archaeology* published a thematic issue on biomolecular archaeology in 1993, there was a notable enthusiasm for the new methods emerging. Studies on ancient proteins as well as ancient lipids were discussed, but the major interest was focused on ancient DNA and archaeogenetics. Several areas that could benefit from ancient DNA analysis were brought forward; among them were ancient diseases, human dispersal, residual analysis of artefacts (genetic studies on ancient artefacts), and domestication studies. Today, 13 years later it is evident that many of the promising ideas brought forward was wishful thinking, and few of them have actually been carried out successfully. In this paper, I will talk about the main obstacles associated with ancient DNA analyses, give some examples on how to overcome these problems and present new methodological methods emerging in the field. The two main limiting factors in the ancient DNA field proved to be DNA preservation and contaminating modern DNA, both were larger problems than anticipated. Nevertheless, DNA from prehistoric material has served as a good tool in studying ancient fauna and ecology, and in some cases the ancient data have been useful in answering archaeological questions. Up until recently, studies on ancient DNA was limited to mitochondrial fragments, and the development was focused on increasing the sample size rather than on increasing the amount of genetic data per individual. This is not only because of the usefulness of this marker; it is also an advantage to work with multicopy fragments when approaching degraded material. Limiting factors in the field can also be attributed to the ancient specimens themselves and the extracted ancient DNA from it. DNA sampling being a destructive method sometimes mean small amount of sample only allowing for a certain amount of amplifications, limited to a certain marker. The possibility to multiplex ancient amplifications, and to target hook sequences is currently being explored and would allow getting more data from less material and less extract. Moving on from mtDNA, SNP technologies could provide the possibility to type variable nuclear DNA in degraded material, mainly due to the minimal fragment size of these systems. SNP technologies applied to ancient DNA do looks promising, and if successful, will provide an opportunity to study genes under selection in historic time. Attempts have also been made to retrieve random ancient nuclear DNA. There are several drawbacks with such methods, however they could yield large amounts of informative autosomal DNA. Further, there has also been development on the issue of contaminating DNA and the possibility of getting at authentic ancient human DNA. So far it has been complicated to argue authenticity for such material since a conclusive way of distinguishing contamination from authentic ancient human DNA have been lacking. In order to overcome

this problem quantitative differences between contamination and ancient DNA is being studied, this may eventually provide a way to authenticate ancient human DNA. Although the expectations on ancient DNA studies as expressed in the 1993 biomolecular issue of *World Archaeology* were set too high, recent developments may have opened up to some of the original ideas. After a decade of methodological development and higher stringency, we might again dare to thoroughly exploit the serial dimension only provided by DNA from ancient samples.

# Compound-specific determination of $\delta D$ values of animals for the reconstruction of ancient diet

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Stable hydrogen isotope ratios, termed  $\delta D$  values, in precipitation vary according to season, climate, latitude and altitude. It has been found that the  $\delta D$  values of meteoric water are passed on, without significant fractionation, to the stem water of plants growing in a given region and that the  $\delta D$  values of animal lipid depend on diet and drinking water; these  $\delta D$  values should be preserved in archaeological pottery. Recent advances in instrumentation have introduced the possibility of compound-specific stable hydrogen isotope analyses, using gas chromatography – thermal conversion – isotope ratio mass spectrometry (GC-TC-IRMS), which have the potential to characterise ancient saturated fatty acids according to animal type, diet and geographic origin, with significant palaeodietary applications. We are developing a protocol for the compound-specific hydrogen isotope analysis of fatty acids preserved in clay pottery. Here we present the results of investigations into the extent of hydrogen exchange observed in fatty acids absorbed onto clay; showing that although exchange of up to two hydrogen atoms occurs it tends to have little effect on the  $\delta D$  value of the fatty acid, which is typically altered by less than experimental error (typically  $\sim 5\%$ ). Further, exchange during cooking and simulated burial of fatty acids absorbed onto clay pottery does not alter the  $\delta D$  value of the acid by more than  $\sim 8\%$ . We also present  $\delta D$  values for modern fatty acids of marine, and ruminant and non-ruminant mammals reared on known diets in continental and non-continental locations, with geographical (more depleted at continental sites) and trophic level (more enriched at higher trophic level) trends observed. The difference in  $\delta D$  values between marine and ruminant animals is of the order of  $35\%$  for stearic acid.

# Testing Bayesian models of natural selection and sequence damage using ancient DNA data sets

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Recent studies have suggested that purifying selection acts over time scales that are relevant to studies of Pleistocene fauna. It is important to take the effects of this selection into account when analysing ancient DNA data sets, because it can have a substantial impact on estimates of divergence times and of population sizes. In particular, it can lead to significant overestimates of the age of the most recent common ancestor of a clade. The presence of sequence damage has a similar effect. We introduce a model in which the mutation rate is allowed to decay backwards in time, reflecting the action of purifying selection. As a consequence, the mutation rate will be higher at the tips of the tree, where there is an abundance of transient polymorphisms. This model is implemented in a Bayesian phylogenetic framework in the programme *BEAST*, and permits estimation of the mutation rate, proportion of sites under purifying selection, and half-life of slightly deleterious mutations, along with other evolutionary parameters. A second model is presented, which estimates the proportion of polymorphisms that result from damage (or sequencing errors) on the basis of the inferred positions of mutations on the phylogenetic tree. If unaccounted for, this spurious variation can inflate estimates of the mutation rate. These two models are tested on a wide range of ancient DNA data sets, including bears, cattle, cave lions, and bison. The results of these analyses indicate that the impact of purifying selection is considerable, and that the frequency of sequence damage/error is relatively high, at about 1 per 1,000 bases. New estimates of divergence times and population sizes for Pleistocene fauna are also presented.

# A comparative study of GC/C/IRMS and *irm*-LC/MS: Implications for palaeodietary studies in archaeology

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Compound specific approaches to the analysis of archaeological bone collagen are becoming increasingly relevant to palaeodietary studies (Corr et al. 2005). Controlled feeding experiments suggest collagen isotope values are largely influenced by dietary protein, but vary with respect to the amount of protein and the isotopic values of lipids and carbohydrates, particularly in moderate to low protein diets (Howland et al. 2003). Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC/C/IRMS) and Isotope Ratio Monitoring Liquid Chromatography Mass Spectrometry (*irm*-LC/MS) enable the isotopic analysis of single amino acids, including essential (diet derived) and non-essential (synthesised or diet derived) amino acids, which may provide alternative insights to bulk collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (i.e. non-protein dietary components). We compare these systems as a means to investigate the reproducibility of compound specific  $\delta^{13}\text{C}$  measurements on modern and archaeological collagen. GC/C/IRMS samples are analysed as N-pivaloyl-l-propyl esters (NIP) (Metges et al. 1996) and N-acetyl-l-propyl esters (NAP) (Meier-Augenstein 2004). Sample derivatization is not required for the new *irm*-LC/MS system (Thermo Electron IsoLink<sup>TM</sup>) and collagen hydrolysates are analysed directly (McCullagh et al., submitted for publication). We report the  $\delta^{13}\text{C}$  variation of essential and non-essential amino acids and compare them to bulk collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values obtained via continuous flow isotope ratio mass spectrometry (CF-IRMS). Archaeological bone collagen from a variety of contexts is analysed and the utility of compound specific approaches to palaeodietary and palaeoenvironmental studies is discussed.



# Stable hydrogen isotopes and trophic levels in archaeological material

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The stable hydrogen isotope ratios of bone collagen of archaeological material has been measured to test whether a trophic level effect can be observed, as demonstrated in earlier studies of modern fauna. Humans and animals have been studied from four archaeological sites: Yarnton and Eton Rowing Lake in England, Huari in Peru, and Balatonszarszo in Hungary. Across these disparate environments a trophic level effect in hydrogen isotopes is observed, and stable nitrogen and carbon isotope ratios provide further isotopic patterning. Calculations based on amino acid physiology experiments and models help elucidate the contribution of food and drinking water to the observed hydrogen isotope signal.

# New developments in proteomics for the analysis of archaeological organic residues

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Artifacts from coastal archaeological sites in Alaska and Canada were selected at the National Museum of Natural History, Washington DC and residues were analyzed to determine their lipid and protein composition. Cooking residues, derived from marine fat and meat, were trapped as a charred layer adhering on the surface of the pots. Methods to analyze proteins on archaeological artifacts have in the past given few reliable results; proteins are difficult to handle and are denatured and modified when they are processed, especially by heating. Post-burial exposition and ageing lead to further degradation and modifications. A methodology derived from new developments in proteomic analyses have been applied to search the residues for proteins related to marine mammals. After extraction and denaturation of the proteins, the extracts are digested with trypsin, an enzyme that cleaves the proteins into peptides at specific amino acids. The mixtures were analyzed by MALDI-TOF (Matrix Assisted Laser Desorption Ionisation-Time of Flight) and ESI-MS<sub>2</sub> (Electrospray Ionization-Tandem Mass Spectrometry) and proteins identified by searching databases for related peptide sequences. These techniques were successfully applied to archaeological residues sampled from about 25 clay sherds and stone lamps and a peptide fingerprint was characterized and compared with similar fingerprints from modern sea mammal tissues. In addition, the clay matrix of a potsherd from Point Barrow, an area where blood was used to temper the clay, was tested and revealed the presence of hemoglobin  $\beta$ -chain. Although proteins have been degraded by previous heating, proteomics analyses of archaeological samples proved that peptides could still be identified several hundred years after their processing. A few proteins have so far been identified, offering promising perspectives to further characterize specific species.

# New investigations into the Pistacia resin aboard the Ulu Burun shipwreck

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Resin within Canaanite amphorae from the Late Bronze Age shipwreck discovered off the coast of southwest Turkey at Ulu Burun has previously been identified as *Pistacia* spp. Due to the volume of resin held within the numerous amphorae and its widespread and large scale use it is commonly held that these vessels transported the resin itself as a commodity. However, it has been proposed that the Ulu Burun jars contained wine, with the resin used as a sealant on the interior surfaces. To attempt to resolve this question, we have analysed a further five samples of pistacia resin from the Canaanite amphorae using a range of analytical techniques which have used in the past for the analysis of wine residues: Spot tests, IR, and HPLC-MSMS. As well as the archaeological samples, we have analysed modern samples of pistacia resin, leaves and fruit (the fruit is commonly embedded in the resin in the Canaanite jars) to determine the effectiveness of each technique and to exclude the possibility of false positive results. In addition to the analysis of wine we will also present detailed analysis of the terpenoids (GC-MS) for the purpose of further molecular characterisation of the resin. In addition, we have used bulk stable isotope analysis in comparison to a data set of similar resins to attempt to identify the resin source.

## A Bio-archaeological GIS database

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Our goal is to construct a small-scale geographic map that displays ancient DNA preservation conditions above and beyond a simple cold-hot scale.

Therefore, we will develop a GIS-based database that displays the geographic position of archaeological sites and the yielded samples that were subjected to bio-molecular analysis.

We will characterize each sample by its individual properties, i.e. dating, kind of tissue, species, size of animal, gross morphological preservation of sample, etc. Then, we will categorize the quality of ancient DNA retrieval and compare this with various other variables available for the sample. Our main goal is to put the DNA amplification success rate in the context of geographic data, such as latitude, longitude and elevation as well as precipitation data, temperature and geological properties of the site. Furthermore, the database will contain other data related to bio-molecular preservation: collagen content, %C, %N, C/N ratio, thermal age, SAXS value, splitting factors, and various others.

# Digging for ancient DNA: The forensic excavation of a (post)medieval cemetery in Eindhoven, the Netherlands

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During a small test excavation in 2002 on the site of the St. Catharine's Church in the medieval town-centre of Eindhoven (the Netherlands) numerous well preserved human skeletons were discovered. In one of the burials (a ten years old child in the chancel of the church) ancient DNA was discovered. The discovery of DNA had provoked an intriguing archaeological problem. It is the question of how one should deal with, and possibly preserve, an archaeological record with ancient DNA. For the site in Eindhoven there are a number of actual threats, which will diminish the chance to find ancient DNA in future. Probably the only way of preserving this information was to excavate it now it is still available. Ultimately a large scale and complete excavation was done in March 2005-July 2006. During the excavation the remains of the medieval brick church were found. The human remains have outnumbered the expectations. Some 500 individuals were found both in the church as on the former churchyard, which were generally well preserved in situ. They date from the thirteenth till in the nineteenth century. Most individuals were sampled on DNA in a forensic way. The samples will be investigated to gather information on kinship, sex, origins and genetic defects through the ages. Extra ancient DNA samples were collected to be investigated for medical purposes.

# Dietary patterns and genetic variability of the Imperial Roman population at Velia (Salerno, Italy, I-II cent. AD ): A combined biomolecular approach

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The ancient city of Velia (called Elea during the Greek period) was under Roman control since the second century BC where it thrived as an important port and economic centre. Immediately adjacent to the ancient port is a large graveyard which contains over 230 burials (necropolis of *Porta Marina*). Graves are inhumations with a broad range of different tomb types (*busta*, cremations, monumental tombs, *cappuccina* etc.). Considering the history of Velia and the archaeological evidences, it could be assumed that the cemetery contains the remains of people of different social status and possibly immigrants to the city. The aim of this research project is to reconstruct the genetic variability, the demographic history and the diet of the population of ancient Velia using a combined biomolecular and paleobiological approach. Together with the study of the paleodemographic profile, the analysis of ancient mitochondrial DNA has been used to estimate the genetic heterogeneity of the population. This study has been combined with stable isotope analysis of bone collagen to investigate the dietary variability. Strontium isotopes determination in teeth of selected individuals is also planned to further identify recent immigrants within the population. We expect that this combined approach could highlight the historical reconstruction of the life of this peripheral Imperial Roman society. Here we report on the initial results from this study and outline a new methodological rationale for the simultaneous extraction of both DNA and collagen from bone.

# Introgession of European Aurochs into imported domestic cattle population?

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The first domestic sheep, goat, pigs and cattle appear during the Neolithic. Our previous studies potentially show that the origin of European domestic cattle can be traced back to the Near East and that no independent domestication of European aurochs can be observed. Nevertheless, there is still debate on possible subsequent crossbreeding between imported cattle and wild oxen. So far only mitochondrial data from Neolithic samples have been investigated, meaning the interpretation is restricted to maternal lineages. Thus, only limited conclusions about crossbreeding can be made. Recently, Götherström et al. (2005, Proc. R. Soc. B 272) analyzed Y-chromosomal *loci* and demonstrated a significant influence of male aurochs lineages, especially in modern Northern European cattle populations. Our goal is to show whether this occurred as early as the Neolithic period or not until a later time. We will also present different patterns of male and female introgession of European wild oxen into Neolithic domestic cattle populations.

# A tale of two settlements: Kinship analysis of Middle Bronze Age cemeteries

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The transition from the middle to late Bronze Age in Greece is characterised by a shift of power from island civilisations (the Cycladic, Aegean and Cretan ‘superpowers’) to a central mainland civilisation (the Mycenaean). The processes behind this power shift are unknown. Middle Bronze Age mainland Greece was relatively poor but there is evidence of long distance trade links. The later influence of Mycenae is due in part to extensive kinship links with other cities (such as Agamemnon’s brother Menelaus in Sparta). Were these links established in the middle Bronze Age or later? As part of a project to discover the kinship links within and between the two rich grave circles of Mycenae, a wider understanding of the genetic makeup of Bronze Age Greece is needed. Therefore, two distant settlements have been assessed using mitochondrial DNA, Y-chromosomal SNPs and STRs and genomic STR targets. Kouphovouno near Sparta and Antron in Lamia are distant and poor enough not to have had direct links with one other. Here the results of the surveys of Kouphovouno and Antron will be presented and the social implications discussed.



# Organic geochemical characterisation of soils from archaeological sites: analysis of sediments from the shell midden at West Voe, Shetland

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Systematic analyses by gas chromatography-mass spectrometry (GC-MS) of the lipid content of soils and sediments across archaeological sites for the identification of source specific biomarkers and their relationship to artefactual and ecofactual finds has rarely been undertaken. Organic geochemical research in this area has largely focused upon the characterization of faecal input for the identification of specific latrine deposits and manuring practices; with some work also carried out on the potential of soil organic matter to contaminate ceramics. Lipids in soils and sediments contain characteristic biomarkers potentially enabling the identification of source materials including anthropogenically influenced inputs. However, the relationship between lipid content, environment and depositional processes is extremely complex and an understanding of patterns of lipid preservation is essential to archaeological interpretations seeking to identify such anthropogenic activities. This study seeks to apply a broad, multiple biomarker approach to these issues by assessing the distribution and relative abundances of the free lipid fraction in sediments from the shell midden at West Voe, Shetland and to determine their relationship to site formation processes. A range of modern soil samples, vegetation, molluscan and faecal materials will also be analysed to assist in input identification and to provide a database of potential contaminants of archaeological materials routinely analysed by GC-MS. (208 words)

# Isotopic evidence of salt-marsh economies? Modern and archaeological case studies from Britain

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Salt marshes are among the most biologically productive ecosystems in the world, serving as a transitional zone between the land and sea. They are inhabited by characteristic ‘salt loving’ plants (or halophytes). Prior to regimes of banking and land claim, areas of salt-marsh were far more extensive than today and represented a vast, exploitable resource. Halophytes (both C<sub>3</sub> and C<sub>4</sub>) have distinctive nitrogen and carbon isotope ratios – in particular, they are significantly enriched in <sup>15</sup>N compared to other fully-terrestrial grazing plants. In the Severn Estuary (south-west England), direct archaeological evidence - including fossilized footprints - indicates salt-marsh use by humans and animals from the Mesolithic to the Post-Medieval period and zooarchaeological mortality profiles imply specific herding strategies. However, the precise dynamics of the seasonal relationship between humans, herded-animals and the salt-marsh is not known and no quantitative method exists for exploring the extent of salt-marsh and estuarine grazing in the past. This study uses modern and ancient faunal remains to explore the relationship between halophyte chemistry and the bone chemistry of halophyte-grazed herbivores with the aim of assessing the potential of using stable isotopes to indicate past coastal herding strategies. This involves carbon and nitrogen stable isotope analysis of bone and dentine collagen of archaeological faunal material (bovids and ovi-caprids) from Bronze Age contexts at the Severn Estuary sites of Redwick, Peterstone and Brean Down. Isotope analysis of modern wool and bone from organically-reared salt-marsh-grazed lamb from the Steart Flats, Somerset, is undertaken to provide additional information and for comparison to the archaeological data. We aim to develop a new method for indicating patterns of salt-marsh exploitation and coastal subsistence economies in the past.

# Proteomics on archaeological samples: A useful approach?

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Ancient DNA investigation is the most common way to retrieve genetic information from the past. Nevertheless in some cases an alternative approach was represented by the study of proteinaceous remains. The most of this work was based on the use of immunological methods, but the recent development of proteomics techniques, based on high throughput mass spectrometry systems, allowed an increase in sensitivity and specificity. Although previous studies reveal that proteins appear prone to degradation in archaeological contexts, it has been recently demonstrated that mass spectrometry can be fruitfully used to recover ancient protein sequence. Moreover according to recent models the interaction between proteinaceous residues and soil colloids can apparently protect these residues from microbial and chemical degradation processes. HPLC analysis of some organic residues and soil samples from different archaeological contexts provide an indication of the preservation of proteinaceous residues in archaeological samples. However, methodological improvements to optimise each analytical step are still required. In particular attention is being focused upon (i) the extraction method, (ii) reliability criteria, (iii) data analysis, the latter through the implementation of algorithms specifically development for the investigation of degraded samples.

# Reconstructing prehistoric Korean subsistence activities using stable isotopic analysis

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Neolithic cultures of Korea (B.C 8000-1500) are characterized by both hunter-gatherer and shifting cultivation societies, yielding enormous shell midden sites and the unique *Jeulmun* pottery for food storage. While the transition from hunter-gathering to agriculture occurs during Middle Neolithic, based on the presence of domesticated foxtail millet and agricultural stone tools, it is believed that primary subsistence activities continued to emphasize deep-sea fishing, shellfish gathering, and hunting. Furthermore, even though rice agriculture was introduced to Korean peninsula in the Bronze Age (B.C1500-300) with wet-field feature sites that indicates paddy field rice-farming, it is believed that the people continued to grow millets, barley, legumes, together with collecting and hunting terrestrial mammals and seafoods. To better understand the prehistoric subsistence activities in Korea, analysis of carbon and nitrogen isotope analysis has been applied to archaeological bone samples. Specifically, carbon isotope values in bone collagen are examined to detect potentially the advance period of millet and rice agriculture in Korea and nitrogen isotope from bone proteins is used to detect the exploitation of terrestrial mammals and seafood during the Neolithic. Isotope ratio mass spectrometry (IR-MS) has been applied to bone samples from one Neolithic site, the Dongsamdong (n=20), and one Bronze Age site, Nukdo (n=50). The carbon and nitrogen isotope values suggest that the Neolithic Dongsamdong shell midden inhabitants are heavily dependent on marine resources for their dietary protein, even though there are many terrestrial mammal bones such as deer and wild boar. Preliminary results suggest that marine protein obtained from fish and sea mammals played a significant role for food resources in Neolithic coastal regions of prehistoric Korea, in conjunction with the terrestrial mammals.

# Dietary and geographical fingerprint of ancient British Columbian glacier body through molecular and isotope characterisation of bone and skin lipids and amino acids

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Molecular fingerprinting and compound-specific carbon isotope analysis was applied to individual lipids and amino acids extracted from bone and skin samples of a glacier body in a forensic-style investigation, undertaken to elucidate where the individual originated and how much time he endured such inhospitable surroundings prior to his death. Kwaday Dän Ts'inchí, the finest preserved ice body unearthed in North America, was recovered from a retreating glacier within the Tatshenshini–Alsek Park in British Columbia on August 14, 1999. High temperature-gas chromatographic analysis of both tissues revealed a distribution consistent with degraded human lipids (C<sub>12:0</sub>, C<sub>14:0</sub>, C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>18:0</sub>, and C<sub>18:1</sub> fatty acids, cholesterol, 10-hydroxyhexadecanoic acid and 10- and 12-hydroxyoctadecanoic acids). Despite his discovery 80 km from the coast, the bone sample revealed unusual compounds almost exclusively observed in marine organisms: C<sub>20:1</sub> and C<sub>22:1</sub> fatty acids and their microbially-activated oxidation products, 10- and 12-hydroxyeicosanoic acid and 10- and 12-hydroxydocosanoic acid, respectively, and the marine biomarker isoprenoid trilogy of phytanic, pristanic and trimethyltetradecanoic acid. It was hypothesised that the bone lipid distribution originated from the consumption of a marine-enriched diet throughout life. Comparison of bone and skin cholesterol  $\delta^{13}\text{C}$  values indicated a deviation to include terrestrial dietary sources prior to death. This result was congruent with tissue amino acid  $\delta^{13}\text{C}$  values, in particular  $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$  values, which were higher and more marine-like in bone ( $15.6 \pm 1.7\text{‰}$ ) than skin ( $12.7 \pm 1.7\text{‰}$ ). Hence, both the molecular and isotopic composition of individual lipids and amino acids are consistent with the consumption of a marine-enriched diet throughout life (bone signature), followed by the inclusion of C<sub>3</sub> terrestrial foods in the months prior to death (skin signature). This individual evidently spent the majority of his life in a coastal environment followed by either an occasional or single journey inland in the final months of life.

# Cultural Continuity or Change?: lipid residue analysis of Romano-British mortaria

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Whilst extensive work has already been undertaken on non-visible lipid residues from pottery sherds from Neolithic, Bronze Age, Saxon and Medieval sites in Britain, little work has thus far been published on lipid residues from the Roman period. Although the introduction of Roman ingredients into Britain is well-documented from macrobotanical, technological and literary evidence, the appropriation of Romanised foodways and techniques of food preparation at individual sites within Britain is more difficult to recognize. Mortaria, which are shallow, gritted and spouted dishes, are a novel vessel type which is often perceived as synonymous with a 'Roman' diet and continental traditions of food preparation, although the exact function is unclear. Here, well-established techniques of Gas Chromatography (GC) and GC/Mass Spectrometry have been employed in order to determine whether lipid residues are recoverable from this specialised vessel type, and to ascertain the possible origins of these fats and oils. Analysis of >140 sherds has shown that appreciable lipids ( $>5\mu\text{g/g}^{-1}$ ) are well-preserved at a range of sites (e.g. Vine Street, Leicester: 46%; Faverdale: 50%; Wroxeter: 56%; Rutland villas: >90%; Piercebridge: 87%). Sherds from waterlogged deposits at Piercebridge also show unusually high lipid concentrations, with 44% yielding greater than  $>100\mu\text{g/g}^{-1}$  and nearly 20% yielding  $>1000\mu\text{g/g}^{-1}$ . These residues contain evidence of leafy vegetables, along with high proportions of saturated fatty acids and sterols of animal origin (e.g. cholesterol and its degradation product  $5\alpha$ -cholestanol) which are rarely preserved in potsherds, indicating that anaerobic deposits may offer particularly favourable preservation conditions. Compound-specific isotopic work will further elucidate the origins of these animal fats and allow us to compare the use of this vessel at different site-types within the geographical and temporal span of Romano-Britain.

# Natural toxins and narcotics in ethnographic and archaeological collections

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The analysis of amorphous organic residues from archaeological and ethnographic museum collections provides direct evidence for the use of natural materials, which can help enrich interpretations of traditional societies. The value of this approach is increasing, driven by advances in extraction and analytical techniques that permit the analysis of an ever-wider range of compounds. This project demonstrates the application of modern instrumental techniques of chemical analysis, in particular pyrolysis Gas chromatography-Mass spectrometry (GC-MS) and Liquid chromatography-Mass spectrometry (LC-MS) to trace organic residues from archaeological and ethnographic artefacts perceived to be associated with the use of pharmacologically active natural products. The aim is to demonstrate that chemical residues of narcotics and toxins may survive in archaeological and ethnographic contexts. The research is based on developing analytical procedures capable of resolving and identifying the specific plant alkaloids using artificially aged refined alkaloids, modern botanical reference standards, ethnographic and archaeological samples. A knowledge of the decay mechanisms will then make it possible to analyse heavily degraded archaeological samples effectively. The ability to identify trace residues of high value organic commodities in archaeological and ethnographic artefacts will enable a greater understanding of the diversity of use, cultural significance and long distance trade of such materials to be more fully investigated.

# Distinguished by diet: The Neolithic Pitted Ware Culture in the Baltic Region

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The Neolithic Pitted Ware Culture, archaeologically defined primarily by its characteristic pottery, occurring at coastal locations roughly 3500–2500 BC, has been the subject of archaeological controversy for many years. Partly coeval with the Funnel Beaker and Battle Axe Cultures, both associated with agriculture, the Pitted Ware Culture has been suggested to represent, for example, places for ritual pot destruction, coastal activities by farming groups, ideological transformations of the Funnel Beaker Culture, hunter–gatherers and pig herders, or seal hunters with a common cultural identity – interpretations which are partially or entirely incompatible with each other. Human and faunal remains from one mainland and five island sites in the Baltic Region, archaeologically assigned to the Pitted Ware Culture, were analysed: Korsnäs on the Swedish mainland, Västerbjers, Ire and Visby on Gotland, Köpingsvik on Öland (Sweden) and Jettböle on the Åland (Ahvenanmaa) Islands. Human bone and dentine collagen representing more than 100 distinct individuals were subjected to stable carbon and nitrogen isotope analyses. The analyses were supported by extensive stable isotope data from Korsnäs and Västerbjers fauna, as well as radiocarbon dates of both human and faunal remains from most of the sites. Based on these data, it is argued that the Pitted Ware Culture in fact did represent a distinct group of people, seal hunters who were in contact with farming groups, but did not practice themselves any agricultural activities.



# First stable isotopes data of late glacial hunter-gatherers from Balma Guilanyà site (south-eastern Pre-Pyrenees, Spain)

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Stable isotopes analysis of carbon and nitrogen was performed on bone collagen extracted from one human individual and six animal remains found at the Late Upper Palaeolithic site of Balma de Guilanyà (Catalonian Pre-Pyrenees, Spain). Conventional and AMS radiocarbon determination from associated charcoal from the site place the occupation within the Bölling/Allerød interstadial. No stable isotopes studies have been carried out on individuals of this period from Spain so far, and only few have been made in the rest of Europe. Human remains recovered at this site are scarce and fragmentary, consisting only of 14 teeth, small skull fragments, and a handful of postcranial bones represented by three phalanges, one scaphoid and a left radius fragment. The teeth belong to a minimum of four individuals, but the postcranial bones probably belong to a single individual. The analysed fauna correspond to meal refuse and are thus fragmentary and badly preserved. Four samples are from herbivores (red deer and wild goat), there is another herbivore sample of undetermined specie and the other belongs to a rabbit. If we take the values of the whole group of large herbivores, leaving out the rabbit sample, we obtain a mean of 1,92‰ for  $\delta^{15}\text{N}$  and of -19,87‰ for  $\delta^{13}\text{C}$ . The values from the human radius from Balma de Guilanyà are  $\delta^{15}\text{N}$ = 6,55‰ and  $\delta^{13}\text{C}$ = -19,98‰. By comparing the human values with those of their contemporaneous fauna, we come to the conclusion that the studied individual consumed primarily animal protein of terrestrial origin. Low  $\delta^{15}\text{N}$  values of both animals and human samples were also found by other authors in contemporaneous samples from northwest Europe. They detected a significant  $\delta^{15}\text{N}$  drop in collagen from bones during the last glacial stadial, followed by a rise at the beginning of the Holocene. This phenomenon had not been observed in the Mediterranean region until now, and it was thought that it was restricted to northern Europe.

## Lipid Residues in African Neolithic and Iron Age Pottery

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Using established techniques, potsherds from archaeological sites in Kenya, and Niger were analyzed for lipid traces using GC and GC-MS. Those containing appreciable lipids were further analyzed using GC-C-IRMS to determine the possible origins of the animal fats recovered from these sherds. Potsherds from three sites in Kenya (Gogo Falls, Siror and Jangili Rock Shelter) and one site in Niger (Adrar Bous) were analyzed. Forty-six percent of the Gogo Falls sherds contained appreciable traces of lipids in concentrations ranging from 6  $\mu\text{g g}^{-1}$  to 221  $\mu\text{g g}^{-1}$ . In addition, twenty-five percent of the Siror sherds, twenty-three percent of the Jangili sherds, and fifty-three percent of the Adrar Bous sherds contained lipid residues with mean concentrations of 22  $\mu\text{g g}^{-1}$ , 19  $\mu\text{g g}^{-1}$ , and 13  $\mu\text{g g}^{-1}$ , respectively. In most of the lipid-bearing sherds, fatty acid distributions high in palmitic ( $\text{C}_{16:0}$ ) and stearic ( $\text{C}_{18:0}$ ) acids indicated that the residues were of animal origins. Some sherds also contained plant waxes or beeswax (indicated by the presence of wax esters). An unusual triacylglycerol distribution, tentatively assigned a non-animal origin, was present in one sherd from Gogo Falls. Another sherd from the same (inland) site had high levels of eicosanoic ( $\text{C}_{20:0}$ ) and docosanoic ( $\text{C}_{22:0}$ ) acid, a distribution previously only seen in lipid residues of marine origin; since sixty-six percent of the faunal remains at the site are lacustrine fish, this seems likely to be the origin of these long chain fatty acids. Stable isotope values ( $\Delta^{13}\text{C}$ ) indicated that the  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$  fatty acids, when compared to British animal reference fats, were consistent with a non-ruminant adipose origin. GC-C-IRMS of fats from African animals will provide a more solid reference set to which to compare the  $\delta^{13}\text{C}$  values of the residues recovered from the sherds.

# Ancient DNA from soils and sediments: A new tool for reconstructing past environments

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Genetic analyses of ancient DNA (aDNA) from sediments and soils have potential as tools for reconstruction of past environments, even in the absence of macro-fossils. Ancient DNA extracted from New Zealand Cave Sediments and Arctic Permafrost Soils was examined for the presence of Mammal, Bird and Plant species. The presence of these species was then used to infer past environmental change and in addition, quantitative PCR was used to test for potential leaching of extra-organismal DNA in temperate soils. DNA sequences of extinct biota characteristic of the pre-human environment were recovered from sediments up to 3300BP (including two well-dated volcanic tephra) in two sites in the North Island of New Zealand, as well as from two permafrost sites in Alaska, and one permafrost site in Russia. The Arctic sites date from 10000 to approximate 7000BP, a period dramatic climate change during which many species of megafauna became extinct. The aDNA in sediment records the deposition of physical remains of organisms, or their ejecta and this DNA persists after macroscopic traces vanish. Ancient DNA is unlikely to be deposited from aerosol, so sediments will not record the presence of organisms present around a site, if their remains were not physically incorporated in it. Despite these restrictions, ecological research will benefit greatly from this new methodology, which can record former patterns of biodiversity, how vegetation and faunas have changed in time and space, and the effects of natural disturbances of past ecosystems.

# Evaluating Neandertal genetics and phylogeny

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The retrieval of Neandertal (*Homo neanderthalsensis*) mitochondrial DNA is thought to be among the most significant ancient DNA contributions to date, allowing conflicting hypotheses on modern human (*Homo sapiens*) evolution to be tested directly. Recently, however, both the authenticity of the Neandertal sequences and their phylogenetic position outside contemporary human diversity have been questioned. Using Bayesian inference and the largest dataset to date, we find strong support for a monophyletic Neandertal clade outside the diversity of contemporary humans in agreement with the expectations of the Out-of-Africa replacement model of modern human origin. From average pairwise sequence differences, we obtain support for claims that the first published Neandertal sequence may include errors due to post-mortem damage in the template molecules for PCR. In contrast, we find that recent results implying that the Neandertal sequences are products of PCR artefacts are not well supported, suffering from inadequate experimental design and a presumably high percentage (>68%) of chimeric sequences due to “Jumping PCR” events. (max. 300 words)

# Organic geochemical evidence for pine tar production in Middle Eastern Sweden during the Roman Iron Age

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Several funnel-shaped features of unknown function were discovered at excavations related to a new stretch of the highway E4 in eastern middle Sweden 2002-2003. These features could be sub classed into two categories; large funnel shaped pits dated to 600-1100 AD (Vendel period - Viking Age) and small funnel shaped pits dated to 240 - 540 AD (late Roman Iron Age - Migration period) respectively. Soil samples were analysed for diterpenoids derived from abietic acid (mainly retene, abietic acid, dehydroabietic acid and methyl dehydroabietate) by Gas Chromatography - Mass Spectrometry (GC-MS) in order to test the assumption that the features might be connected to tar manufacturing. For comparison, samples from historically known tar and charcoal production features were analysed. The biomarker methyl dehydroabietate could be identified in several of the soil samples from the funnel shaped pits. The resinous fraction in the larger funnel shaped features was very similar to the historical tar and charcoal production features, while the composition in the small funnel shaped pits was more concentrated to retene and methyl dehydroabietate. We suggest that these features have been used for pine tar production which makes the smaller funnel shaped features the oldest known tar production features in Europe.

# Palaeodietary study on Xiaojingshan Site, China: Stable isotopic evidence

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Xiaojingshan Site was dated to 8000 years ago in the lower part of Yellow River.

Palaeodietary reconstruction will have great importance to get the information on human diet in Chinese early Neolithic. Carbon and nitrogen isotopes in 17 human bones were measured. The mean  $\delta^{13}\text{C}$  value ( $-17.77 \pm 0.34\text{‰}$ ) showed that C3 foods were dominant with C4 foods supplementary. The mean  $\delta^{15}\text{N}$  value ( $8.99 \pm 0.54\text{‰}$ ) indicated most protein in foods came from animals. Although this site is located in the area where C4 crop, *ie*, millet, is dominant in Yellow valley, the millet agriculture did not develop highly and hunting still was the main lifestyle. Compared to isotopic data in Jiahu site, a site 8000 years ago in Yellow River, more millet was consumed in the diet.

# Social differentiation and diet in Early Anglo-Saxon England: Stable isotope analysis of archaeological human and animal remains from Hampshire

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The diet of the early Anglo-Saxons (410-700 AD) in Britain presents archaeology with a moderate amount of circumstantial information about diet (faunal/literary), but not a concrete understanding of the process involved in production and consumption during this Dark Age period. The current study will look at the stable isotopes,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , recorded in bone collagen of both humans and the animals they were eating in order to gain a better understanding of the complexity of this pattern which has only been studied on a small scale at the Anglo-Saxon Cemetery of Berinsfield, Oxfordshire (Privat *et al* 2002). The stable isotope evidence will be compared to six different types of recorded archaeological evidence for Anglo-Saxon inhumations. The evidence can be divided into two basic categories: dietary evidence reflecting biological criteria (sex, age and height/stature) and dietary patterns representing cultural differentiation (grave goods, body position, and grave orientation). A sampling strategy focusing on animals and humans is being developed in the research programme as it is necessary to use the faunal remains from consumed animals to gain an isotopic baseline. The isotopic record of humans cannot be properly reviewed/interpreted without knowledge of the isotopic pattern of the foods they were eating. The results for three Anglo-Saxon sites near the modern city of Winchester are presented in order to demonstrate the suitability of stable isotopes to differentiate social groups. The sites are King's Worthy (Worthy Park), Winnall II, and Abbot's Worthy located in the Itchen River valley. Isotopic changes through time highlight how dietary shifts reflect greater social developments in Anglo-Saxon culture with the introduction of Christianity. An unexpected  $\delta^{13}\text{C}$  isotopic shift between animals and humans (+2‰) will show how different food intake can affect the isotopic patterns of humans. The identification of this pattern would not have been possible without the large scale sampling of animal remains.

# Identifying determinants of DNA preservation and characterising bacterial degradation of bone

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The recovery of ancient genetic information holds great promise for the understanding of human phylogeny and past migration, and has the potential to contribute significantly to other areas, such as analysis of emerging diseases, and molecular anthropology. To date however, comparatively little is known of what determines DNA preservation in archaeological remains. The aim of our project will be to identify the processes of diagenetic change in bone that are determinants of DNA preservation. Microbial alteration of the bone will cause loss of original aDNA, may cause contamination with bacterial DNA, and increases the risk of contamination with recent DNA (Gilbert 2005). Identifying organisms altering bone, would facilitate the identification of contaminating DNA, as well as shed light on the mechanisms of early post-mortem degradation of bone. We will, using quantitative real time PCR, try to shed light on the authentic DNA template quantity and quality, as well as the amount and species origin of micro-organismal DNA present in samples. Finally, we will attempt to correlate the preservation of DNA to histological characterization of bone, identifying bacterial alteration, within the same sample. The comparison of characteristics at the morphological, histological, and molecular level is critical for the development of predictive criteria, which could eventually serve as an objective framework within which to evaluate claimed results of DNA testing as authentic, or contaminant/artefact.



# Intra skeletal stable isotope variation

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Stable isotope analyses of archaeological human bone samples are routinely used for reconstructing the dietary history of individuals or populations. This project focuses on intra skeletal variability of the stable isotopic compositions ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of human bone collagen. This study comprises 74 well preserved skeletons of both adults and juveniles from a medieval cemetery in Denmark. Sampling strategy was based on the well documented archaeological and historical records indicating that these individuals were a homogeneous population, consuming a similar diet throughout their lives. From each individual a sample from the femoral diaphysis and rib was selected, and furthermore a sample of the petrous part of the temporal bone was taken from 58 of the individuals. Our results show that the petrous bone has an isotopic signal that differs significantly from both femur and rib values within the single skeleton. Conversely, only minor variation was found between femur and rib. The intra skeletal variations may reflect differences in turnover rates among the skeletal elements. The inner periosteal layer of the petrous bone is formed in utero and does not undergo remodelling after the age of two, whereas the rib and femur have a continuous turnover rate of 5 and 10-20 years respectively. Our results show that the petrous bone may be a new useful bone element and a supplement or a proxy for teeth in the analysis of migration patterns as it reflect the diet in foetal stage and early years of life.

# Ancient DNA: Killing exogenous DNA with hypochlorite

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A major problem tightly associated with human ancient DNA is the immense risk of contamination with extraneous human DNA. When teeth are used as DNA source common decontamination procedures involve wiping of the surface with bleach or even immersing the teeth in bleach. Also submersion of powdered tooth in bleach has been used. We have investigated the various ways of decontaminating material from teeth with bleach. Teeth extracted from skeletons from Danish Medieval and Icelandic Viking sites were used. To see how deep hypochlorite penetrates into teeth that were in bleach, whole teeth were treated with radioactive chloride ( $Cl^{36}$ ) under similar conditions. Testing of the cut surfaces showed that in all cases radioactive  $Cl^-$  was abundant in the core of the teeth, even after washing with water. We therefore assumed that treatment of powdered pulp directly by submersion in hypochlorite for a few minutes would be a more reproducible way of decontamination. Powdered pulp from 5 individuals was treated by suspension in 50% commercial bleach (~ 2% hypochlorite) for 5 min followed by extensive wash with water. Ancient DNA was then extracted, PCR amplified, cloned and sequenced. In each case the haplotypes observed were identical with those observed for the same individuals using only external decontamination of the teeth. Thus, it was possible to treat powdered pulp with hypochlorite and afterwards extract authentic DNA. To study the efficiency of hypochlorite decontamination, whole teeth were suspended in a solution of a 414bp PCR product (1,000,000 copies). However, even without hypochlorite treatment no amplicons were detectable in the pulp. Results of current decontamination experiments where powdered pulp is contaminated by suspension into a solution of amplicons will be communicated.

# Sulphur isotope analysis from two Viking Age cemeteries: A comparison between a Christian and heathen site in Sweden

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The well-known Viking age proto-town Birka situated on the island Björkö in lake Mälaren, Sweden has been the focus in many Early Medieval Scandinavian studies. The wide range of burial types, all non Christian, on the island among other things have lead to a discussion on where the buried people came from, since it is obvious they did not live on the island. As a sharp contrast to the heathen Birka is the cemetery in Björned, Ångermanland in Northern Sweden. All burials in this Viking Age/Early Medieval cemetery are Christian. Björned is considered to be connected to the east/western trade between the eastern Baltic and Norway. Where does this Christian enclave come from? Did they come as missionaries settling in a heathen country? There are contemporary heathen cemeteries in the vicinity. We have approached these issues using stable isotopes analysis of carbon, nitrogen and sulphur all on collagen. Despite slight differences in, a mainly terrestrial based, diet seen in the carbon and nitrogen isotopes there is a large variance in sulphur values. This can only be explained by a different geographic origin for both the individuals from Birka and the individuals from Björned.

# A new approach on the origins of horse domestication in the Iberian Peninsula: Genetic analysis of Bronze Age horses from El Portalón de Cueva Mayor site (Sierra de Atapuerca, Burgos, Spain)

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The time, the location, and the process of the domestication of *Equus caballus* have been studied in different disciplines. Particularly, the process and the geographical area are some of the questions that have risen controversial debate. Genetic analyses on mitochondrial DNA (mtDNA) of extant horses support the multiregional domestication hypothesis, pointing at the Iberian Peninsula as a centre of independent horse domestication. Archaeological analyses suggest that the domestication of the horse in the Iberian Peninsula took place during the Calcolithic – Bronze Age period. In this study we present mtDNA of 20 ancient Iberian horses, twelve belonging to the Bronze Age, and show how Iberian wild horses contributed to shape the extant Iberian horse breeds.

# Genetic analysis of historic cereal landraces aids in understanding the spread of agriculture across Europe

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The *Domestication of Europe* consortium project explores the spread of agriculture in Europe by means of phylogeographic analyses of modern landraces of barley (*Hordeum vulgare*) and emmer wheat (*Triticum turgidum* subsp. *dicoccum*). Data derived from historic and archaeological specimens are also being included in the study to determine the extent of “overstamping” of ancient genetic patterns. Historical cereal landrace material that pre-dates industrial agriculture and modern plant breeding (dating from early mediaeval times until the mid 20<sup>th</sup> century) is more readily available than archaeological material, and generally has better DNA preservation. Historic material has been collected from a number of sources across Europe, including herbarium collections, museums and historic buildings. DNA extraction protocols are being developed for cereal samples of different ages and from various contexts, and DNA preservation is being assessed by the recovery of PCR amplicons of different lengths based on the upstream region of the High Molecular Weight Glutenin locus in wheat. Preliminary results show that good quantities of DNA can more readily be extracted from grains than from vegetative material. Hence materials such as thatch and daub, where grain is rarely available, are of limited use. The effect of smoke (in the case of smoke blackened thatch) and mercuric chloride (in the case of some herbarium specimens) on DNA preservation is being evaluated.

# No signs of an ancient Scandinavian domestication event in Middle Neolithic dogs

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Mitochondrial DNA (mtDNA) studies on modern dog breeds display a complicated picture of their maternal relationship. Four to six more or less well defined haplogroups have been identified, and the number of domestication events has been suggested to be about the same as the number of haplogroups. There is, however, one haplogroup that seem to be more distinct than the others. This haplogroup (called hg D or clade II), comprise mainly of the Scandinavian breeds Jämthund and Norwegian Elkhound, and is therefore believed to represent an ancient Scandinavian domestication. If this is the case, the haplogroups should be present in pre-historic Scandinavian dogs. To test the antiquity of this haplogroup and a possible Scandinavian domestication event, we selected bones and teeth from 28 middle Neolithic (~2500BC), 3 Iron Age and 6 Medieval dogs. The material derived from mainland Sweden and also from three Baltic Sea islands. DNA was extracted with a silica-based method and extracts were amplified/quantified using real-time PCR. Two 100 bp fragments from the dog mtDNA D-loop region were targeted using different sets of primers. PCR-products were sequenced and the sequences were assigned to haplogroups. The majority of the samples yielded amplifiable DNA and all belonged either to haplogroup A or C. Since none of the sequences clustered with hg D/clade II, we found no support for continuity between modern and ancient Scandinavian dogs. We can see two possible explanations for our results. The first one is that the distribution of hg D/clade II does not originate from a Scandinavian domestication event, but is rather a breeding artefact. The second one is that hg D/clade II do actually represent a Scandinavian domestication event, but one that is younger than 4500 years.

# A new method for amino acid $\delta^{13}\text{C}$ analysis of bone collagen using *isotope ratio monitoring-LC/MS*

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Methods for the isotopic analysis of individual amino acid from archaeological bone proteins have become increasingly useful in palaeodietary studies in recent years. However, these methods have been mainly reliant on Gas Chromatography Combustion Isotope Ratio Mass Spectrometry (GC/C/IRMS) which until recently has been the only online technique available for compound specific isotopic analysis. This paper will introduce a new technique for the online carbon isotopic analysis of *underivatized* amino acids using an HPLC method coupled to a Thermo Electron IsoLink (*irm-LC/MS*). This has advantages over GC/C/IRMS analysis as it requires only minimal sample preparation and no derivatization of amino acids. We report baseline separation of 15 of the 18 proteogenic amino acids and their isotope values with an average standard deviation of 0.18‰ (n=6). In addition amino acid  $\delta^{13}\text{C}$  values from archaeological bone collagen will be presented to show the method's applicability to palaeodietary studies and wider applications.

# Experiences with aDNA analysis of human remains from the Danish Medieval, Viking and Iron-Ages

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The present results are obtained as part of the project “Denmark’s Genetic Past”, with the aim of describing population heterogeneity, maternal relationships, family and tribal patterns, population affinity and migration patterns. Teeth were used as DNA source and all pre-PCR work was carried out in a dedicated aDNA-extraction lab. All analyses were performed on three teeth each by different investigators. DNA was extracted from pulp, short segments were PCR amplified, cloned and sequenced. Aligned sequences were analysed for post-mortem derived damage patterns. At this stage, we have haplotyped approximately 35 individuals from four locations dating to Medieval, Viking and Iron Ages (ca. 600-2,000 YBP). Only teeth from well-preserved individuals were used, and for the majority of the individuals tested it was possible to obtain concordant results for all three teeth. A surprising amount of haplogroup diversity was observed, indicating that our forefathers were as different as we are today. The haplogroup I was overrepresented. Three haplotypes, not observed amongst present day ethnic Scandinavians, were also observed; this may be evidence of connections with populations from far away regions (U2, Pre-HV, N1a). To investigate the nature of pre-laboratory contamination, in particular the stage at which extant human DNA enters ancient samples, a study is currently being conducted of remains from a Viking site, Galgedil (1,200 YBP), where we have been able to extract teeth from 9 individuals under controlled conditions directly at the excavation site. Two teeth were taken using the before mentioned measures and a third tooth from each individual was retrieved after the material had been handled, washed and labelled by the archaeologists. These remains are unique since humans have not touched them for the past 1,200 years and we have been able to haplotype all those involved in the post-excavation manipulation of the remains.



# 1500 years of human diet in York – the evidence of stable isotopes

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Carbon and nitrogen stable isotope analysis of bone collagen is a well-established technique for reconstructing past diets, however, many applications are still restricted to relatively small case studies of chronologically and geographically diverse sites. We present the results of a large-scale (~450 samples) diachronic study of a single site by isotope analysis, namely York in Northern England from the Roman period to the 18<sup>th</sup> century. The isotope data clearly show a remarkable dietary breadth and variation in diet, especially in the use of marine resources, between different time periods as well as between individuals of different social status. The chronology and implications of these findings are discussed with special reference to the fish bone evidence. We aim to demonstrate how large-scale investigations can maximise the potential of isotopic studies and contribute new evidence that is inaccessible by more traditional archaeological sources.

# Molecular damage in ancient DNA

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It is known that post-mortem damage represents one of the main drawbacks to obtain DNA from an ancient specimen. Post-mortem damage generally involves breaks and cross-links in the double-strand DNA molecule that leads to enzymatic replication inhibition. Spurious DNA nucleotide changes spontaneously come out due to deamination and hydrolytic depurinitation. Then, the determination of the real DNA sequences on the specimen under analysis is not straightforward. In addition to, ancient and/or degraded DNA is extremely prone to contamination, which is many times difficult to monitor. Here we have analyzed the sequence variation at the mitochondrial DNA (mtDNA) molecule in an ancient population of Bronze Age from Atapuerca (Spain) which has allowed us to explore the DNA damage. It has been proposed that at least 1,000 copies of initial template DNA is needed to guaranty the reproducibility of sequence results; below this number, the resulting sequences are likely to contain artefacts. Our results corroborate this general belief, and reinforce the conclusion that monitoring DNA quantification is crucial when analyzing aDNA. DNA contamination is another major concern because it can imitate sequence damage and also allows jumping PCR between endogenous and contaminant strands. Jumping events will increase the apparent number of damaged sites in cloned sequences by introducing positions that differ between the contaminant and authentic DNA. When the clones are analyzed and the changes are counted, an increase of the C to T changes is detected; therefore, a bias to cytosine deamination follows. On the other hand, it is generally known than the ratio transition:transversion in ancient DNA is of a similar order of magnitude to the ratio detected in natural populations (aprox. 30:1); we detected however that this ratio is lower in our samples (aprox. 10:1). Mutation spectra detected in our samples fits well (specially for hotspots) with the one previously observed by others using ancient DNA or observed in natural populations.

# Palaeoproteomics of mature tooth enamel: Problems and potential

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In the past five years MALDI-TOF mass spectrometry has proven to be an ideal tool for sequencing protein extracts from ancient bones. So far this research has focused on the bone protein osteocalcin which has a good survival record in a range of burial environments (Nielsen-Marsh *et al.*, 2002; Nielsen-Marsh *et al.*, 2005, Ostrom *et al.*, 2006) owing to its affinity for bone mineral, which appears (at least in part) to stabilize the protein (Nielsen-Marsh *et al.*, 2005). However, osteocalcin is a genetically conserved protein and informative phylogenetic studies using osteocalcin sequences will always be limited. Post transitional modifications can potentially offer phenotypic information, but other proteins need to be utilised if MALDI-TOF mass spectrometry is to be exploited fully for ancient biomolecular studies. As well as bone, teeth also survive in the burial environment, and although dentine often fares as badly as bone against diagenesis, enamel, the hardest substance in the body, is widely believed to be the best preserved element in skeletal remains and may provide the best source of proteins in fossil specimens. However, mature, modern enamel contains (at most) only 0.03% of protein in its structure. This very limited amount presents a problem for the development of successful extraction and purification protocols for sequencing enamel proteins from ancient samples. This poster presents results from a series of experiments aiming to extract and sequence mature enamel proteins using a combination of chromatography, gel electrophoresis and MALDI-TOF mass spectrometry.

# The use of DNA from museum specimens in conservation biology: The Scandinavian arctic fox

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In recent years, ancient DNA has frequently been used to address conservation genetic questions. In this study, we used DNA from museum specimens to examine the genetic consequences of a demographic bottleneck in the endangered Scandinavian arctic fox. Variation in the mtDNA control region and five microsatellite loci in the pre-bottleneck population were compared to modern samples from Scandinavia and North Russia. The microsatellite data from the museum specimens was further used to simulate the expected effect of the bottleneck. The results show that the arctic foxes in Scandinavia have lost approximately 25% of the microsatellite alleles and four out of seven mtDNA haplotypes. The results also suggest that the genetic differentiation between North Russia and Scandinavia has doubled over the last 100 years. However, the level of heterozygosity was significantly higher than expected from the simulations. This demonstrates that analyses of DNA from museum specimens give an important temporal dimension to conservation genetic studies of extant populations.

# Genetic analysis of ancient humans from Libyan Sahara

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About 10,000 years ago the Sahara region appeared as a verdant landscape attracting animals and vegetation from southern latitudes and human groups as well. Instability of climate conditions up to the second part of the Holocene led to an arid phase, the Mahla event, which forced human groups to adapt their food security and settlement systems. Wadi Tanezzuft Valley, in the Acacus region (south-western Fezzan, Libyan Sahara), was one of the major river systems which offered support to people over many generations during the dry period. DNA was extracted from bone and teeth samples of 27 skeleton excavated in the Wadi Tanezzuft Valley dating to Pastoral and Garamantian period. Following stringent criteria proposed for ancient DNA authentication, we have analysed polymorphic sites within the hypervariable region I and II (HVR-I and II) and other SNPs in the coding region which are diagnostic for major branches in the mitochondrial DNA (mtDNA) tree. Phylogeographic analysis indicate a genetic continuity among human groups who succeeded one another during the Pastoral period and describe a scenario of continuous movement and intermingling of people in a rapidly transforming Saharan landscape. Considering the location of this site the success rate of DNA amplification was unexpectedly high. This may be due to the microenvironment within the tombs which in some cases led to desiccation of the corpses. We aim to conduct biochemical and microscopic analysis to further assess the state of bone preservation.

# A full plate – combined spectroscopic studies of solid organic food residues in cooking vessels from the Roman Iron Age

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The molecular characterization of solid organic residues found in association with ancient ceramic cooking vessels can give direct information about the presence of lipids, proteins and polysaccharides in processed food. However, the study of organic residues is primarily focussed on extractable compounds (e.g. lipids, waxes, and resinous materials) preserved as absorbed residues – compounds preserved after absorption into the actual ceramic fabric of the vessel. The alternative approach is the analysis of surface residues – visible solid organic residues preserved as crusts or films adhering to the interior or exterior surface of ceramic vessels. Although, the chemical characterisation of these solid surface residues is analytically complicated by the complexity of the material and limited sample size, there are various methodological arguments for the study of surface residues. Firstly, extraction is a selective technique and the residue may not be representative for the food under study. Secondly, the study of visible surface residues makes it possible to sample only one layer of material, while absorbed residues are a combined deposit of multiple use-phases. And finally, absorbed residues have usually been exposed to a more severe thermal regime (both in time and in temperature) than residues situated on the interior surface of the vessel, which complicates the interpretation of results. The application of various pyrolysis techniques (DTMS) make it possible to characterize both the lower molecular weight, volatile part (including fatty acids, sterols and acylglycerols) and the non-volatile, cross-linked part of a solid sample formed at higher temperatures from native proteins and polysaccharides. Solid state FTIR and the solid-state CP/MAS <sup>13</sup>C NMR spectroscopy provide additional information on the overall chemical characteristics of food residues. This combined solid-state spectroscopic study of solid organic food residues in cooking vessels from the Roman Iron Age, illustrates the technical challenges and shows how some of the subsequent difficulties in archaeological interpretation can be dissected.

# Late Bronze Age diet in Greece

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In this paper we present the dietary variation in Greek Late Bronze Age populations, using stable carbon and nitrogen isotope analysis of human bone collagen. The dietary reconstruction was conducted in five sites, Aghia Triada, Elis, Voudeni, Achaia, Zeli and Kalapodi, Phthiotis and Triada, Rhodes Island. Comparison of our values with European human values with marine diets and with faunal values, resulted in that none of the individuals showed any significant marine input. All five populations present C3 plant and terrestrial animal protein input, but there are differences in the amount of the animal protein use and the access to C4 plant protein. This may indicate that differences between sites are based mainly on the environment. Additionally, we show that social status differences do not influence a group's or an individual's access to the basic food sources.

# Using ancient DNA to detect changes in the population structure of *Plasmodium vivax*

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DNA sequencing has been a useful tool in aiding our understanding of ancient diseases. This study aims to determine the extent to which it is possible to use ancient DNA sequencing to study changes in the molecular diversity of *Plasmodium vivax*, as well as to gather information on changes in parasite structure and virulence in medieval and post-medieval England. Historical records and literature from the time suggest a change in parasite virulence through the centuries, with a peak occurring in the 1600's. This study will investigate these claims and draw upon both archaeological and biological evidence to discern a more complete picture of a changing parasite. A silica-based method was used to extract ancient DNA from bone samples collected from notoriously marshy areas of southeast England. PCR amplification targeted the 18S rRNA gene of *P. vivax* and the human mtDNA hypervariable region 1 to assess the survival of DNA in the archaeological samples. Ancient malarial DNA was extracted and successfully amplified from several samples thus far and research is continuing to obtain sequence data from additional samples.



## DNA degradation after excavation

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Most ancient DNA studies are performed with fossil samples that have been stored in Natural History collections. To evaluate whether the storage conditions and/or preparation treatments of “fossil” bones in those collections are adapted to ensure DNA preservation, we compared DNA recovery from two types of bone samples: (1) bones that had not been washed or treated with any chemicals but were kept in the cold (“fresh” fossils); (2) bones that had been washed and treated according to standard procedures and then stored in collections (“old” fossils). In several cases, we obtained authentic DNA sequences from “fresh” fossils but not from “old” fossils from the same archaeological sites. When comparing the success rate of PCR amplification from a large-scale study of 235 bovine bones from 53 different archaeological sites of various depositional contexts, we obtained authenticated amplification products from “fresh” fossils in 33% whereas “old” fossils yielded those products in only 14%. The quantity of 150 bp-long molecules that could be amplified was three times higher when the extracts were made from “fresh” fossils than from “old” ones. Finally, we could amplify DNA only from freshly excavated, unwashed fossil bones of a 3,200 year-old aurochs but not from bones of the same animal that had been stored for decades in a museum. We could determine that during the last 57 years when the aurochs bones were stored in a collection at least as much amplifiable DNA was lost as during the previous 3,200 years of burial (99% in both cases). Thus, we demonstrate that DNA preservation depends not only on the taphonomic processes occurring prior to excavation but also on the treatment and storage conditions that are commonly applied to fossil bones and that can be detrimental to DNA “survival”.

# Early and Late Medieval diet of Italian populations

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During the early Medieval period (300-900 AD) rural southern Italian populations usually preserved a late antique diet, which included grains and small amounts of animal protein coming from secondary products. In the late Medieval (1300-1500 AD) period, urban Italian sites display differential consumption of meat and fish according to social groups. While written sources provide evidence of aggregate Medieval diets, stable isotope analysis provides more direct data about food consumption by specific groups of people and individuals. Isotopic composition of different body tissues reflects the isotopic signatures of the foods consumed during the individual's lifespan. In this manner, carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic analysis of bone and dentin collagen can provide information about constituents of the diet during childhood to the last few years of life. The diet of two Italian populations from the beginning and end of the Middle Ages was studied here using bone and teeth samples. Bones and teeth were collected from 15 individuals at the site of Castro dei Volsci (dated to ca. 600 based on grave goods) and from 35 individuals at Rome (dated to the late 1470s based on archaeological and archival evidence). Statistically significant differences in both carbon and nitrogen isotopes of bone and tooth collagen between these two populations likely results from increased fish in the diet. Moreover, the variability in  $\delta^{15}\text{N}$  values within the Rome population indicates a range in fish consumption at this site, with 7 individuals obtaining significantly higher portion of their dietary protein from marine sources. No difference in mean isotopic composition was observed between males and females. These results are in agreement with the historical documentary evidence.

# Reconstructing African-Caribbean life-history trajectories using C, N and Sr isotope analysis – a case study from Barbados, West Indies

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This poster presents the results of a recently completed isotopic study of the remains of thirty-two captive Africans who lived on the Newton plantation, Barbados during the era of plantation slavery (1650-1820). The methodology largely follows the approach described by Sealy *et al.* (1995) and proceeds by measuring C and N stable isotopic ratios in different skeletal elements in order to track changes in individual dietary regimes that are likely to relate to an incisive life history event. The results of the analyses reveal that nine out of the thirty-two sampled individuals showed a considerable shift of up to 10‰ in  $\delta^{13}\text{C}$  and up to 5‰  $\delta^{15}\text{N}$  values between different skeletal elements, which reflects a drastic change in diet that is likely to have coincided with the enslavement and dislocation of this group from West Africa to the West Indies. Sr isotope analyses using LA-ICP-MS on tooth enamel samples from this group were able to confirm the hypothesis that we are dealing with African-born individuals, and allowed further to make some first tentative suggestions regarding the possible origins of these individuals within Africa.

# Analysis of carbon isotope values of single amino acids extracted from land snails (*Bithynia tentaculata*) using LC-IRMS

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Molluscs from terrestrial archaeological and palaeontological deposits can provide a number of different types of palaeoenvironmental data. One source of data is the isotopic ratio of carbon from the protein fraction of semi fossil shells. Protein makes up approximately 0.1% of the shell by weight and thus contamination, degradation, and sample size are matters of concern when doing such analysis. Analysis of the carbon isotopes of bulk organic matter extracted from shells cannot determine if a sample is contaminated and requires sample sizes in the region of 300-500mg of shell. Furthermore, differential degradation of amino acids with differing isotopic ratios will cause variation in the bulk organic matter values and again this is undetectable. Here we present results of analysis of carbon isotope ratios of single amino acid fractions extracted from the opercula of land snails (*Bithynia tentaculata*). The intra-crystalline fraction of the amino acids was extracted in the same manner as for amino acid racemisation dating. After which the carbon isotope values of single amino acid fractions were analysed using Liquid Chromatography – Isotope Ratio Mass Spectrometry (LC-IRMS). This technique chromatographically separates the amino acids from the hydrolysed sample then converts the sample to CO<sub>2</sub> and measures the isotope values of the CO<sub>2</sub> in one on-line system. This technique has a number of advantages over bulk analysis in that 1.) the amino acid profile can be observed and thus contaminated or degraded samples can be noticed, 2.) duplicate analysis can be made with samples of about 20-30 mg and 3.) individual amino acid isotope values provide more detailed information than bulk analysis. The LC-IRMS technique also has advantages over other techniques employed to measure single amino acid isotope values as the system is fully on-line and there are no derivatisation procedures.

# Demographical aspects of the great migration: Are there age limitations in mitochondrial dating?

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Geneticists infer our African origin from the existence of greater mitochondrial variability in Africa than in any other continent. "This implies that African individuals have had more time to diverge than any other population". However, according to some estimations (that we shall see below), African inhabitants in the year 400 BC represented 11% of the world's population. This means that, or else a lot of more people should have come out of Africa (89%) than stayed, or else very different growth rates are necessary. If we opt for first option, we will be obliged to explain because the geneticists still find the biggest proportion of mitochondrial sequences in Africa. If we opt for second one option, we will need two very different growth rates, one, that grows slowly, for Africa, and other one faster for the rest of the world. If we itemized the growth rates for each continents we found out that the rates need to be faster for each new continent. That in the case of the Americas is five times faster than Africa rate. We believe that small deviations to the general rate could have existed in each continent. But I believe that to think America may have grown five time faster that Africa, and that did it during forty thousand years, does not seem reasonable. We believe that the low demographical growth rate of population in the Pleistocene has to do with this problem. If we base on the hypotheses of Out to Africa model, we will be able to estimate the growth rate during the Pleistocene. But the low demographic growth rate is the cause of other problems: breakability of lineages during the Pleistocene, and modification of the statistic distribution of variability between populations according to its growth rate.

# The composition of some Roman ointments: Evidence for Pliny's Punic wax?

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Organic residues from medicine containers in the collections of the British Museum have been analysed as part of a wider programme of scientific work on Roman surgical instruments. The cylindrical bronze containers were found to contain *materia medica*, ranging from very obvious and extensive remains of ancient medicines to possible minor deposits on interior surfaces. Samples taken from the residues were analysed by gas chromatography-mass spectrometry (GC/MS) to identify lipid and carbohydrate components. These provided evidence for ointments (and in one case possibly pills) consistent with a medical purpose. Ingredients identified included beeswax, fat, conifer resin and gum-derived sugars. Particularly significant were the varied compositions of residues from four sections of a multi-compartment container. In one of these compartments the beeswax seems to have been prepared as the 'Punic wax' described by Pliny. We used Pliny's recipe to prepare Punic wax in the laboratory. This was analysed by GC/MS and the results compared to those from the Roman containers. This paper describes the manufacture process of the Punic wax, and discusses the GC-MS results of both the experimental material and the archaeological material. Punic wax has not previously been reported from medicine residues, although according to Pliny it was favoured for the purpose. More attention has been given to the use of the material in encaustic paints, but the reported compositions do not seem to be consistent with the results reported here suggesting that the material may have been prepared differently for use as a paint medium.

# Tracing selection in cattle through time using single nucleotide polymorphisms

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Studies of ancient DNA hold the potential to unravel processes of selection in the past. Ever since the domestication of the aurochs (*Bos primigenius*) some 10, 000 years ago, cattle (*Bos taurus*) have been a key element of European livestock. During this time cattle have been subjected to different kinds of processes, like selection or genetic drift, all leading to changes in their DNA. They are likely to have been subjected to selection early on during history but the information we have regarding historic cattle breeding and stock management is scarce. Our knowledge is mainly based on osteological analyses which show that medieval cattle were generally smaller compared to modern cattle. In a pilot study targeting three phenotypically associated single nucleotide polymorphisms (SNPs) in 120 ancient cattle remains (600AD-1800AD) and 90 modern cattle from northern Europe we found a significant decline in individual genetic variation before the 18<sup>th</sup> century, thus predating the 20<sup>th</sup> century industrialised breeding. Neutral markers on the other hand don't show any significant change over time, this further confirms the directed selection that late medieval cattle were subjected to.

# Carbon isotope measurements of individual amino acids

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Stable isotope analysis has become an invaluable tool for the determination and measurement of human and faunal palaeodiets. Bulk carbon and nitrogen isotope signatures of bone collagen can enable the distinction between marine and terrestrial diets, the consumption of C3 and C4 plants, and the influence of climatic and environmental factors. However, the individual amino acids each possess a unique isotope signature, which is determined from its parent compound and affected by metabolic routing. Bulk carbon isotope analysis of bone collagen represents the average value for these 18 different amino acids, which are also present in differing proportions. Glycine, a non-essential, metabolically active amino acid is present at a high proportion in collagen. Thus, its isotope value can often dominate the bulk carbon isotope measurement and mask the isotope signal from essential amino acids, present at lower proportions. The isotope signature from essential amino acids is thought to be derived directly from dietary protein and therefore, by measuring individual carbon isotope values a more direct and accurate assessment of diet may be obtained. Carbon isotope measurement of individual amino acids currently requires either long preparative techniques or procedures which add carbon molecules. However, recent developments in isotope instrumentation, Liquid Chromatography – Isotope Ratio Mass Spectrometry (LC-IRMS), now enables direct on-line carbon isotope measurement of individual amino acids. Using this technique amino acids from hydrolysed collagen are chromatographically separated using a strong cation exchange column followed by a reverse-phase column; this is coupled to the newly developed Thermofinnigan Isolink preparation unit where the amino acids are chemically oxidized, resulting in the production of CO<sub>2</sub>. The CO<sub>2</sub> is then directed to the on-line IRMS, where carbon isotope ratios are determined. We will present results from our current ongoing projects which include samples obtained from controlled feeding experiments, and marine and terrestrial ecosystems.



# Molecularly imprinted polymers: A new approach to the purification of biomolecules for isotopic analysis

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Stable isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of biomolecules such as bone collagen provides important palaeodietary information, such as the presence of  $\text{C}_4$  plants or marine foods in the diet. In addition, radiocarbon dating ( $^{14}\text{C}$ ) of these biomolecules allows for determination of the absolute age, assisting in building chronologies at archaeological sites. The use of these techniques requires uncontaminated samples, a challenge being addressed in part by compound-specific isotope analysis. This method involves the separation and isolation of single compounds which are then analyzed. If the compound is chosen correctly, diagenetic contamination can be significantly reduced or eliminated entirely. Typically these procedures involve complicated and time-consuming chromatographic separations, but molecularly imprinted polymers offer a selective and straightforward way to separate a particular target molecule. The preparation of molecularly imprinted polymers involves the use of a template molecule, typically the molecule of interest or a structural analogue, around which a highly crosslinked polymer is made. Removal of the template molecule results in a rigid polymer support containing cavities that fit, and therefore retain, the molecule of interest. Highly selective separations of the target molecule typically result when the polymer is employed as the chromatographic stationary phase. We have been engaged in the preparation of a molecularly imprinted polymer that will be selective for 4-L-hydroxyproline (hyp), an amino acid that is prevalent in bone collagen but rare elsewhere in the environment. Isotopic analysis of hyp often provides more accurate isotope measurements than bulk collagen. We are working toward the development of a highly selective separation procedure for hyp that will allow its easy isolation from hydrolyzed bone collagen. This poster will present the use of a covalent imprinting technique to prepare a hyp-specific molecularly imprinted polymer.

# Early Holocene population dynamics in European brown bears

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Climatic changes have had great influence on the distribution and behaviour of different species. The Quaternary, was the period with major climatic events and high population dynamics. According to glacial refugia theories, during the ice ages, several species migrated to southern Europe, when great part of northern and central Europe was covered with ice sheets. These species settled in three suggested glacial refugia: the Iberian, Italic and Balkanic Peninsulas. We studied phylogeographic patterns in brown bears using ancient DNA, to provide genetic evidence of geographic isolation supporting glacial refugia existence perhaps, for other animal groups, including hominids.

# Bronze Age toilet habits, and a hearth that went missing: On the use of micromorphological samples at excavations of prehistoric settlements

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In 2005, micromorphological samples were taken at five archaeological sites in Østfold, Norway. Thin sections were made from the samples at the University of Ghent, Belgium. Dr. Scient. Barbara Sageidet at the Archaeological Museum in Stavanger has analysed the thin sections. This paper will focus on our experience with the use of micromorphological analyses in connection with the excavation of prehistoric houses. Does the method have potential to shed new light on the organisation of prehistoric households and farmsteads, or does it just provide us with some “new wrapping”? The paper will focus on samples taken from two sites with prehistoric settlement in Sarpsborg, Østfold. The first site contained, among other things, several Bronze Age rock carvings and the remains of a two aisled Bronze Age house. In connection to this house, a dark, ill-smelling layer was exposed. Two micromorphological samples were taken from the layer. The two samples indicated that the dark layer consisted of garbage remains from the house, and traces of different household activities were identified. One curiosity is the presence of *nightsoil*, a combination of urin and ashes from the hearth. The thin section analysis indicated that the house had been an ordinary Bronze Age farm house, shared by animals and people. The second site contained, among other things, the remains of a three aisled house from the migration period. From a structure interpreted as the remains of an oven or hearth, in the center of the house, one micromorphological sample was taken. The thin section analysis indicated that this interpretation of the structure was wrong. It suggested instead that the structure had been used for drying plants. The two cases examined show how micromorphological analyses can be used to interpret the consistence and function of structures and layers exposed during excavation of prehistoric settlements.

# Population genetics of early domesticated horses from Europe and Asia

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Analysis of mitochondrial DNA shows that genetic variation in a given prehistoric horse population is comparable to that found in modern horses. Horse bone samples from Scythian kurgans (7 - 8th century BC) compared with sequences from an existing database of more than 600 living horses reveal an unexpectedly high genetic diversity within the old Scythian horse population. This leads to the conclusion that horse breeds with a variety of ancestral lineages existed in the distant past and that a large number of wild lineages were involved in the domestication process. Additionally, various samples from pre-domestication and incipient domestication time periods from Europe and Asia are analyzed. By contrast, similar analyses of mitochondrial DNA have shown that modern individuals from cattle, goat and pig breeds are much less genetically diverse than their ancient forbearers. This would suggest that the domesticated horse had ancestors in many places, implying that domestication occurred in many areas and likely over a long period of time.

# Isotopic variation in UK alpacas: Testing an approach for application to Peruvian textiles

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The habitat of domestic camelids in South America is generally focussed on rangeland grazing/ foddering in the highlands. Of the two domesticated species, llama (*Lama glama*) have traditionally been considered as pack animals, whilst the smaller alpaca (*Lama pacos*) with its finer coat has been exploited for wool. Camelid wool isotope signatures are defined by a combination of the fodder source, soil characteristics, elevation and water source. A pilot study was used to investigate the utility of stable light isotope signatures in camelid wool in order to refine issues of textile raw material origins for archaeological applications and greater understanding in conservation practice. Modern alpaca fibres from known location, altitude and geology in the United Kingdom were analysed for stable light isotopes of carbon and oxygen. Wool and fodder samples as well as relevant biographic data were obtained from alpaca breeders belonging to one of the two main organisations – The British Alpaca Society and the British Camelid Association. Isotopic data derived from the wool and fodder samples was georeferenced using conventional GIS software and plotted against elevation. The data was compared against current GIS models for oxygen isotope variation in the UK concerned with the UK maritime climate and geological variation. This work has widespread and fundamental application to research questions concerning the production economics of textile raw materials in ancient societies, such as whether textiles from coastal Peru were manufactured from either locally-sourced camelid wool, or wool imported from the highlands.

# Some aspects of palaeodemography of the Stone Age burials at Zvejnieki

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The Zvejnieki Stone Age complex in northern Latvia includes one of the most significant hunter-fisher-gatherer cemeteries in Northern Europe. The burials of 317 individuals were excavated here by Francis Zagorskis in the years 1964–1970. Radiocarbon datings from the cemetery range from 8150 to 4190 BP. Two settlement sites have been identified close to the cemetery: Zvejnieki II (Mesolithic) and Zvejnieki I (Neolithic). 139 individuals could be included in the estimation of palaeodemographic indices. Since hunting, fishing and gathering remained the main economic basis during both of these periods, the changes in demographic statistics are small. In the Mesolithic and the Neolithic, there is a marked prevalence of male burials at the cemetery, exceeding females by a factor of 1.8–2.2. In the Middle and Late Neolithic, compared with the Late Mesolithic, the proportion of child and juvenile burials has fallen by half; there is a slight increase in male life expectancy, earlier commencement of reproduction and a reduction in female life expectancy. It should be noted that the demographic changes at Zvejnieki in the Middle and Late Neolithic (5500–4200 BP) cannot be linked to the transition to a food producing economy, since hunting, fishing and gathering remained the main mode of subsistence.

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